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QUANTITATIVE STUDIES ON INTRACELLULAR RESPIRATION

III. Relation of the State of Nutrition of Paramecium to the Rate of Intracellular Oxidation

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It is a well known fact, since Rübner's (1) work, that the rate of respiratory metabolism in mammals is markedly increased after ingestion of food, particularly of protein, while during starvation the respiratory metabolism in general decreases (2). Direct experimental data on respiratory metabolism in invertebrates when subjected to starvation is, however, quite meagre (3), (4). The data in this and the following papers are so far as I know the first to appear on the relation of nutrition to respiration in any of the protozoa. The experiments were carried out on a pure line of Paramecium caudatum.

Several investigators (5) have used the degree of susceptibility to cyanide as an indirect method for the purpose of determining the relative "rates of metabolism" of many different organisms under different external conditions and states of nutrition. This method according to these investigators (5, p. 72) depends for its usefulness upon the assumption that cyanide in appropriate concentrations inhibits the metabolic processes in cells, in particular the oxidations. For Paramecium the assumption that potassium cyanide is a specific inhibitor of oxidations is certainly not valid since it has been clearly shown in the previous paper that the oxidations continue practically unchanged often for many hours, depending upon the concentration of the cyanide, until cytolysis takes place.

The relation of the state of nutrition of Paramecium and Didinium to their resistance as measured by their survival time in potassium cyanide solutions has recently been carefully worked out (6). These experiments definitely showed that the resistance as measured by the survival time in the cyanide solutions increased or remained at a high level when food was ingested by Paramecium and Didinium, while starvation of these animals led to a marked decrease in the survival time in cyanide. It is hardly conceivable that acclimation to cyanide as suggested by Child (5, pp. 72–73), could play any rôle in increasing the survival time in cyanide solutions under the conditions of these experiments. The results with the concentrations, n/75-n/85, employed in these experiments must therefore be classed with those of the "direct method." From this we should expect that the starved Paramecia and Didinia would have a *higher* "rate of metabolism" than their fed sisters. What are the facts?

It is very doubtful if the rate of oxidations as measured by carbon dioxide production or oxygen consumption can even approximately be used as a measure of the rate of total metabolism in a cell for it has never been shown that the speed of such processes as hydrolyses and changes in colloidal constitution are correlated to the speed of oxygen consumption or carbon dioxide production. However, if one finds any advantage in speaking loosely of "rate of metabolism," then perhaps one may use the rate of intracellular oxidation as the best available measure in our present state of knowledge. In any case, the safest method for determining the rate of oxidation is by direct measurement of the oxygen consumption or carbon dioxide output.

The following experiments are reported from among a considerable number, all of which agree in showing that the rate of oxygen consumption is much greater in a cell which has recently taken food than in one which is starving, hence the *opposite* to what would be expected if decrease in resistance to strong solutions of potassium cyanide is an index to increase in rate of oxidations.

That the resistance to cyanide or to any other toxic agent is in some way correlated to some condition of the cell is of course not thereby denied. In fact, difference in resistance of different cells under identical external conditions must be due to some kind of difference between the cells.

EXPERIMENTAL

Two methods were adopted for testing the question. For the sake of clearness the actual procedure employed in the first method will be given by describing that used in the experiment, the results of which are given in table 1, and from which the curve, figure 1, was constructed.

Thirty-six bottles of equal volume—137 cc.—were filled with tap water which had previously come into equilibrium with air in respect to temperature and oxygen content. One cubic centimeter of a concentrated suspension of Paramecium gradually transferred to, and washed two

TABLE 1

Suspension of a pure line of Paramecium caudatum left for thirty-six hours in clear native hay infusion, then centrifuged, transferred slowly from native hay infusion to tap water and finally washed in tap water two times. Thirty-six bottles of 137 cc. each, filled with tap water. Three were used as control blanks. To each one of the remaining 33 bottles was added 1 cc. concentrated Paramecium suspension. Dissolved oxygen given in cubic centimeters thiosulfate. Temperature 21 ± 2°C.

BOTTLE NUMBER	BLANKS ANALYZED AT ONCE	1 CC. PARAMECIUM ADDED TO EACH BOTTLE ANALYZED AT END OF											
		Analyzed at once	10 hours	20 hours	30 hours	40 hours	50 hours	60 hours	70 hours	80 hours	90 hours	106 hours	114 hours
	cc. thio.	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*
1	5.6	5.55	4.10	3.60	3.15	2.60	2.10	1.80	1.60	1.0	0.50	0.10	0.05
2	5.7	5.50	4.30	3.50	3.10	2.50	2.10	1.65	1.55	1.02	0.55		
3	5.7	5.50	4.40	3.50	2.90	2.70	2.15	1.75	0.80(?)	1.02			
4		5.45						1.85					
Average....	5.66	5.50	4.26	3.53	3.05	2.60	2.11	1.76	1.31	1.01	0.52	0.10	0.05

Iodine absorbed by 1 cc. Paramecium, 0.16.

times in tap water with the aid of the centrifuge, was added to all but three of the bottles. These blanks served as controls to determine the amount of iodine absorbed by the 1 cc. Paramecia during analysis.

Four of the bottles containing 1 cc. Paramecium were analyzed for dissolved oxygen at once to determine the original oxygen content and the amount of the iodine absorbed by the Paramecia which was in this experiment 0.16 cc. At ten-hour intervals after the beginning of the experiment as indicated in table 1, sets of two to four bottles were selected at random from among the remaining and analyzed for their dissolved oxygen. Averages of each set were taken and are given in the

table. The variation of room temperature during the experiment which lasted one hundred and fourteen hours or about four and one-half days, did not exceed 2°C. This variation, however, would have introduced noticeable errors in the results if it had not been for the fact that the variations were uniform from day to day and so on the average compensated one another. In other experiments, where necessary, the temperature was kept constant to within 0.2 to 0.3°C. The con-

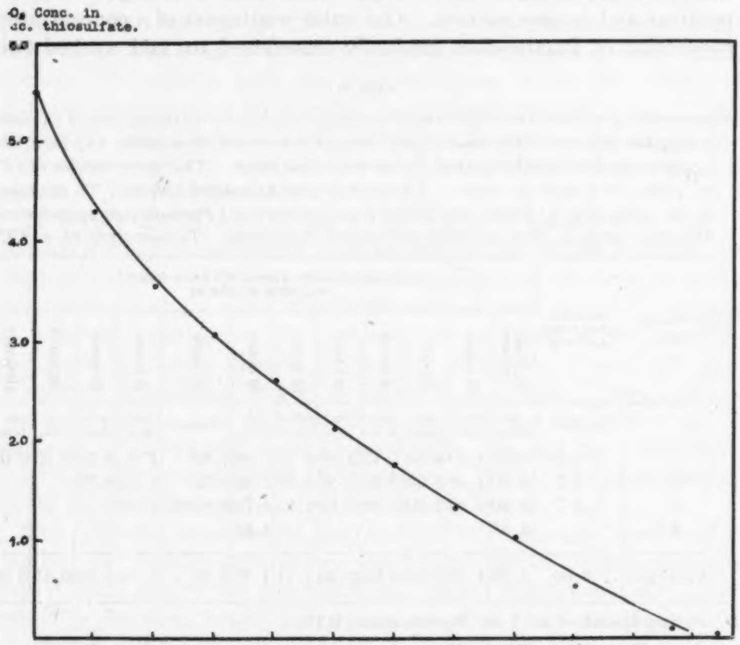

Fig. 1

dition of the Paramecia was carefully watched by means of the binocular to determine if injury or deaths occurred in any of the bottles during the experiment. It often happens that transfer to pure tap water is too sudden or that the centrifuging and mechanical manipulation is too severe. Experiments showing evidence of such unfavorable treatment were discarded and modifications were made in technique so as to eliminate these difficulties in other experiments. It has always been possi-

ble by this means to obtain favorable conditions so that the animals have remained in a perfectly normal and active condition for several days in tap water under conditions of starvation.

The averages of the quantity of dissolved oxygen left at the end of the different time periods are given in cubic centimeters of a thiosulfate solution which was standardized before and after the experiment and found not to have changed. The Paramecia gradually exhausted the supply of dissolved oxygen. The rate at which this supply was used up will be seen from the curve, figure 1. The values on the ordinate represent the average number of cubic centimeters thiosulfate equivalent of dissolved oxygen in table 1, and those on the abscissa the time in hours given in the same table. The rapid fall of the curve during the first twenty hours shows that the rate of oxygen consumption is from two to three times greater at the beginning of the starvation period than later on. The oxygen consumption during the last three days is very nearly directly proportional to the time, since the latter part of the curve is practically a straight line. The close proximity of the points to the curve indicates that the temperature fluctuations that occurred did not, for the purpose of the experiment, appreciably affect the results.

The early period of ten to twenty hours represents the time when the food in the vacuoles and the larger part of the deutoplasmic (food) reserve of the cell becomes exhausted. The protoplasm changes from a gray or dark color in transmitted light to a clear transparent almost colorless condition. A higher initial rate of oxidation immediately after removing from native medium with food to starvation in tap water was also indicated in an experiment in a previous paper (7), figure 1.

During such an experiment the oxygen concentration of course decreases and hence it was at first thought that the above initial high rate of oxidation was due to the relatively high oxygen concentration at the beginning, while the subsequent slower rate was due to the lower concentrations of dissolved oxygen. But this explanation becomes impossible since it has been definitely shown that the rate of oxidations in Paramecium is independent of the concentration of dissolved oxygen (7).

The result of the experiment in table 1 is, however, not in itself conclusive proof that the rapid decrease in rate of oxidation during the first period is due to sudden exhaustion of the major part of the food reserve of the cell for it might be assumed to be due to stimulation of the cell by chemical, osmotic or mechanical environmental changes that neces-

sarily accompany transfer of Paramecium from native hay infusion to tap water. It therefore became desirable to arrange experiments in such a manner that all such objections would be removed by showing that, given two identical lots of Paramecia in the same state of starvation, if one lot is fed and the other left unfed, the fed will consume more oxygen in the same given period of time, under the same conditions, than the unfed. These conditions were fulfilled in the following experiments.

PREPARATION OF THE PARAMECIUM SUSPENSION AND THE METHOD OF FEEDING

Filling the bottles with tap water and preparation of the Paramecium suspensions were carried out according to the method described in a previous paper (8). The animals were placed under varying conditions of starvation in clear native hay infusion, and various dilutions of this clear native hay infusion and tap water. Complete starvation occurred in pure tap water after more or less gradual transfer from the native medium.

The duration or degree of starvation in each experiment is indicated in the descriptions of the tables which follow.

The yeast suspension which was used as food was prepared as follows. One-third of a cake of fresh Red Star or Fleischmann's compressed yeast was suspended in 50 cc. of tap water. A few cubic centimeters of this suspension were boiled in a test tube for two or three minutes. The suspension was shaken to prevent clumping and then quickly cooled. This procedure, of course, kills the yeast and thus prevents consumption of oxygen by the yeast when it is afterward added to the bottles containing Paramecia.

The sample of killed yeast was now washed in tap water two or three times with the aid of the centrifuge in order to remove the extractives which had passed into the water during boiling. This step is important since if the extractives from the boiled yeast along with the yeast itself are added to the water of the bottles which contain the Paramecia, bacteria will sometimes multiply quite rapidly during the experiment and therefore will consume an appreciable amount of the dissolved oxygen. This must be avoided. After carefully adding 1 or 2 cc. of Paramecium suspension to each one of the bottles, about three to five drops of the freshly killed and washed yeast suspension were placed carefully on the *bottom* of each one of the bottles, the Paramecia of

which it was desired to feed. All the bottles were immediately stoppered. The Paramecia in the bottles to which killed yeast was added very soon collected on the bottom of the bottles and in an hour or two they were seen to be filled more or less with vacuoles containing yeast The yeast must be carefully placed on the *bottom* of the bottle using a fine long capillary pipette. If such a small quantity of yeast cells is suspended in the water of the bottle the concentration of yeast is too low to enable the Paramecia to "fill up" on the yeast in a short time

TABLE 2

Paramecium starved in one-half native and one-half tap water for forty-eight hours, then transferred to and left in tap water for fourteen hours and finally concentrated with centrifuge and washed twice in tap water just before being used. By count there were approximately 117,000 Paramecium per 1 cc. of the stock suspension. Oxygen given in cubic centimeters of thiosulfate. Temperature 26 ±. 2°C.

BOTTLE	CONTROLS—ANALYZED AT ONCE				ANALYZED AT END OF 9½ HOURS			
	(1) Blanks	(2) 3 drops yeast added	(3) 1 cc. Paramecium added	(4) 3 drops yeast and 1 cc. Paramecium added	(5) 3 drops yeast added	(6) 1 cc. Paramecium added (starved)	(7) 3 drops yeast and 1 cc. Paramecium added (fed)	Remarks
	cc. thio.	cc. thio.	cc. thio.	cc. thio.	cc. thio.	cc. thio.	cc. thio.	
1	4.07	4.01	4.03	3.93	3.98	3.80	2.98	All active
2	4.01	4.00	4.03	3.95	3.97	3.70	3.04	and nor-
3	4.07	3.89	4.02	3.95	4.03	3.75	2.99	mal.
Average O_2 content..	4.05	3.96	4.02	3.94	3.99	3.75	3.00	
Iodine absorbed.....		0.09	0.03	0.11				
O_2 consumed in 9½ hours............					**+0.03**	**0.27**	**0.94**	

(9). This is an important condition for the best success of the experiment. More than three to five drops of yeast suspension should not be added for this is an ample quantity of food and also reduces to a minimum the error in the analysis which is due to absorption of iodine by the yeast. The Paramecia were added to the bottles previous to addition of the yeast in order to avoid disturbing the latter. Recent experiments by Miss Wolf in this laboratory have shown that both boiled and living yeast which has been washed in sterile tap water will serve as a satisfactory food for Paramecium for pure line experiments,

the medium being sterile tap water. The tap water medium and food can be renewed every day or oftener, if necessary, to prevent bacterial growth; thus, so far as can be judged at present, doing away with the usual variability in the medium and nature of the food when hay infusions are used. Growth and cell division go on at a relatively rapid rate and rhythms in rate of cell division as found by Woodruff (10) are plainly evident. Results of these studies will be published at some later time.

TABLE 3

Paramecia removed from cultures and placed for forty-eight hours in native medium diluted to twice its volume with tap water. They were then centrifuged and washed three times during a gradual transfer to pure tap water over a period of thirty-six hours. Temperature 22.5°C.

BOTTLE	CONTROLS—ANALYZED AT ONCE				ANALYZED AT END OF $9\frac{1}{2}$ HOURS			Remarks
	(1) 2 cc. tap water medium added	(2) 3 drops yeast and 2 cc. tap water medium added	(3) 2 cc. Paramecium added	(4) 2 cc. Paramecium and 3 drops yeast added	(5) 3 drops yeast and 2 cc. tap water medium added	(6) 2 cc. Paramecium added	(7) 2 cc. Paramecium and 3 drops yeast added	
	cc. thio.	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	
1	4.60	4.52	4.49	4.38	4.53	4.10	3.79	All living and normal.
2	4.64	4.59	4.35	4.43	4.47	4.07	3.72	
3	4.59	4.57	4.49		4.51	4.14	3.68	
Average O_2 content..	4.61	4.56	4.44	4.40	4.50	4.10	3.73	
Iodine absorbed.....		0.05	0.17	0.21				
O_2 consumed in $9\frac{1}{2}$ hours............					−0.06	0.34	0.67	

Several preliminary experiments were carried out in order to test what seemed to be all possible sources of error. Tables 2 and 3 give the results of two of the final experiments all of which agreed in showing a marked increase in oxygen consumption after feeding. The error from the presence of the yeast and the Paramecium are corrected by determining the iodine absorbed by the yeast and Paramecium separately, columns 2 and 3, and collectively, column 4, in each of the tables 2 and 3. By looking at these figures in the tables it is clear that the sum of the amounts of iodine absorbed by the yeast, column 2, and the

amount of iodine absorbed by the Paramecia, column 3, are practically equal to the amounts of iodine absorbed by the yeast and Paramecia when placed in the same bottle, column 4. This shows that the corrections are satisfactory and that the control as arranged may be fully depended upon to give values which can be used in determining the actual amounts of oxygen present at the beginning as well as at the end, so far as the errors of the analysis due to the presence of Paramecia and yeast are concerned.

In order to determine if the dead yeast cells which were added to the bottles to be analyzed at the end of the experimental period, column 5 and 7, do consume any oxygen during this period, a control containing only yeast, column 5, was analyzed at the end of the experimental period. The average amount of oxygen consumed by this yeast would of course be the difference between the average oxygen contents of the bottles in column 2 and column 5. This is given at the bottom of column 5 of the tables. It will be seen that this amounts to about 0.05 cc. thiosulfate which is about equal to the largest error in titration with careful work. The amounts of oxygen consumed during the experimental period by the starving Paramecia are given at the bottom of column 6 of the tables, and represent the difference between the average oxygen content at the beginning, column 3, and end of the experiment, column 6. They are respectively, for the two experiments, tables 2 and 3, 0.27 cc. and 0.34 cc. thiosulfate equivalent of oxygen. Since the controls, columns 2 and 5, show that no oxygen has been consumed by the yeast, then it will be evident that the amount of oxygen consumed by the fed Paramecia is equal to the difference between the average oxygen content of the bottles in columns 4 and 7. In table 2 this difference is 0.94 cc. while in table 3 it is 0.67 cc. thiosulfate. The average amount of oxygen consumed in table 2 by the starved Paramecia is 0.27 cc. while that consumed by the fed is 0.94 cc. thiosulfate equivalent, that is, the total oxygen consumed in nine and one-half hours under the same conditions except that of food was between three and four times as much by the fed Paramecia as that by the starved in table 2, and in table 3 it was about twice as much. These differences are very much greater than could be accounted for by errors entering into these experiments. The longer the duration of the starvation the greater within certain limits is the difference in the rates of oxidation of the starved and fed Paramecia. Hence, in order to obtain significant differences in such experiments, the animals must be starved for a sufficient length of time. Several experiments not reported brought out this point

very clearly. Furthermore the animals must be washed carefully in tap water to avoid introducing excessive numbers of bacteria. That bacteria did not develop in those bottles containing yeast and so consumed any detectable amount of dissolved oxygen during the above experiments is shown by the equality of the averages in columns 2 and 5 of the tables. To obtain such results it is usually necessary to wash the boiled yeast in sterile tap water as described above, before adding it to the bottles so as not to introduce any dissolved substances which may serve as a source of food for bacteria.

The question arises, was this great increase in oxygen consumption by the fed Paramecia in tables 2 and 3 accompanied by cell multiplication and hence doubling or trebling the amount of living protoplasm during the experimental period of nine or nine and one-half hours? That cell multiplication did not accompany the two- to threefold rise in oxygen consumption after feeding during the above experimental periods becomes evident from the following facts: *a*, In individual pure line cultures in watch glasses, previously starved Paramecia in tap water suddenly fed on boiled yeast usually do not pass through the first division under the conditions of the above experiments until after periods of from twelve to twenty-four hours. The experimental periods were only nine or nine and one-half hours. *b*. Comparative counts of the number of Paramecia in bottles containing the same number of animals at the beginning, the animals of one bottle being fed while those of the other were not, showed that if division did occur during the nine and one-half hours it certainly did not occur to any detectable degree, for the counts of the fed and starved animals at the end of nine and one-half hours were practically the same. Such counts were carried out for the experiments in tables 2 and 3 by a method the details of which cannot be given here. *c*. Examination of the bottles from time to time by the aid of a binocular showed no dividing animals but did show that the cells had eaten the yeast and were in general larger than the starved ones. *From this it must be concluded that the two- to threefold increase in the rate of oxidation accompanied growth of the cell and not cell division in these experiments.* This is furthermore made certain by a consideration of the curve, figure 1 above, which shows that the oxygen consumption was high at the time of removal to tap water and low at the end of twenty to thirty hours. Cell division could of course not account for this result, and since none of the cells died it is evident that the change in the speed of oxidation is in these experiments clearly correlated in some way to growth and starvation processes in the cell.

The results of experiments, tables 2 and 3, similarly support the previous explanation of the early rapid consumption of oxygen shown in the curve of figure 1, namely that this high initial rate was due to the relatively well nourished condition of the cell immediately after removal from native hay infusion as compared to the condition of acute starvation after twenty hours.

Further convincing evidence that the rate of respiratory metabolism in Paramecium after feeding is increased several fold will be given in a following paper on carbon dioxide production. Discussion of the significance of the results will be postponed until all the data have been reported.

The details of experiment and conditions have been given in some detail, if not fully, for the purpose of showing the precautions necessary and the way in which such experiments must be controlled in order to obtain satisfactory data.

SUMMARY

1. When Paramecium is removed from its native hay infusion in a well nourished condition, to a condition of starvation in tap water, the rate of oxidations in the cell decreases simultaneously with the disappearance of the greater amount of the deutoplasmic food reserve of the protoplasm.

2. Feeding boiled yeast to a Paramecium which has been brought into a state of acute starvation by placing it in tap water may increase the speed of the oxidations two or three times its original amount in the acutely starved but otherwise normal active cell. This increase was not accompanied by cell division in these experiments.

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VIII. THE EFFECT OF ADRENALIN UPON THE FATIGUE PRODUCED BY THE INJECTION OF THE FATIGUE PRODUCTS, LACTIC ACID AND ACID POTASSIUM PHOSPHATE

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Fatigue substances were first demonstrated by Ranke (1) in 1865. He noted the depressing or fatiguing action on skeletal muscle of lactic acid, acid potassium phosphate and carbon dioxide.[1]

The production of lactic acid in contracting muscles and in muscles undergoing death changes was demonstrated by Fletcher and Hopkins (2), Fletcher (3) and others. Lee (4) was the first to record graphically the effect of these substances upon the contracting muscles. He observed that weak solutions perfused through the muscle for a long period of time had the same effect as a strong solution in a shorter time. In both cases a depressing or a fatiguing action was observed. This depressant action of sarcolactic acid, acid potassium phosphate and carbon dioxide was observed in both curarized and non-curarized muscles. A dilute solution transfused through the muscle a short time brought about a temporary increased excitability.

More recently Burridge (5) studied the effects of these substances upon muscles which were excited both directly and indirectly. When he used high concentrations of the acids (0.25 per cent) he found a rapid and complete disappearance of the indirect response, followed shortly by that of the direct. Perfusion of normal saline gave an immediate and apparently complete recovery of the direct response but not of the indirect. In a very short time, however, the response to direct excitation disappeared, regardless of whether or not the perfusion was continued or whether or not the muscle was excited.

[1] For a complete discussion of the early literature on fatigue, the reader is referred to an article written by Frederic S. Lee (4).

That adrenalin markedly bettered the height of muscular contraction was demonstrated by Cannon and Nice (6), Gruber (7) and others. Gruber noted that fatigue decreased the threshold irritability of the nerve muscle preparation and that adrenalin increased this decreased irritability. Gruber and Fellows (8) demonstrated that adrenalin counteracted the decreased height of contraction and decreased irritability due to death changes.

In this research we wished to determine whether adrenalin, which possesses the ability to increase the height of contraction and increase the decreased threshold irritability of a fatigued muscle, would counteract the fatigue produced by perfusing moderately strong solutions of fatigue substances through the muscle.

METHOD

The animals, cats, were anaesthetized with ether and then tracheotomized and the administration of ether continued. The tendons of both tibialis anticus muscles were isolated from their insertions and about each was tied a strong ligature. The anterior tibial nerves were isolated and cut and their distal ends fastened in Sherrington shielded electrodes, held securely in place by bringing together and fastening with paper clips the cut edges of the skin.

Both muscles were then prepared for perfusion. The femoral arteries and veins were isolated and cannulae inserted, after which the animal was quickly killed. One limb at a time was used. Perfusion was started in each limb a few minutes before experimentation was begun. The work on the second limb was started eight to fifteen minutes after the animal was killed.

The medium for irrigation was a warm (36.5° to 39°C.) Ringer's solution at a pressure of 85 cm. of water, containing only the oxygen absorbed from the air.

With one loop of cord about the hock and another around the foot just below the fastening of the tendon, the leg was bound to the board. The ligature from the tendon of the muscle, after passing about the pulleys, was fastened to a muscle lever mounted upon a tripod base. The pulleys were arranged so that the muscle pulled in the normal direction. The muscle lever consisted of a piece of light straw 20 cm. in length from the axis to the tip of the parchment paper writing point. The tendon attached 3 cm. from the axis began at the moment of contraction to pull against an initial tension of 60 grams developed in a

coiled spring For the first 2.5 cm. excursion of the muscle lever on the drum, this was increased 35 grams. The spring was attached at the same position on the lever as was the muscle. Muscular contraction was, therefore, magnified about 6.66 times.

The strength of the stimulating current was 0.1 ampere in the primary circuit which was derived from a storage battery. The stimulating current in every case was maximal make and break induction shocks obtained from a knife blade key, propelled by a motor. The rate, 84 times per minute in these experiments, was slow enough to produce vasodilatation in the vessels of the stimulated muscle. Both make and break shocks were used to prevent polarization of the nerve.

Merck's lactic acid (which was found to be inactive) and Mallinckrodt's lactic acid (which was found to be dextro-rotary 6.91° at 22.5°C.) were used in $\frac{M}{15}$ solutions in most of these experiments. Sarcolactic acid was also employed in approximately an $\frac{M}{3}$ solution. This was obtained by extracting Liebig's meat extract with ether after precipitation of the proteins with alcohol. The optical rotation before the evaporation of water was dextro-rotary 0.57° at 23°C. Acid potassium phosphate was used in $\frac{M}{9}$ solutions. Adrenalin was used in the form of adrenalin chloride and of crystalline adrenalin, epinephrin, 1:1000 solution. In all cases the fatigue substances and adrenalin were injected at body temperature into the perfusion fluid in the arterial cannula.

RESULTS

Acid potassium phosphate. We found as did Lee and Burridge that acid potassium phosphate markedly depresses muscular contraction after an initial excitation. In figure 1 at *1*, 2 cc. of an $\frac{M}{9}$ solution of acid potassium phosphate were injected into the perfusion fluid. There resulted from this injection an initial increase of about 3 per cent, lasting for six contractions, followed by a marked decrease in the height of contraction of about 78.5 per cent, accompanied by a slight development of contracture. This marked drop in the height of muscular contraction was followed by a slight recovery. At *2*, adrenalin chloride (0.5 cc. of a 1:1000 solution) was injected. The muscular contractions at first

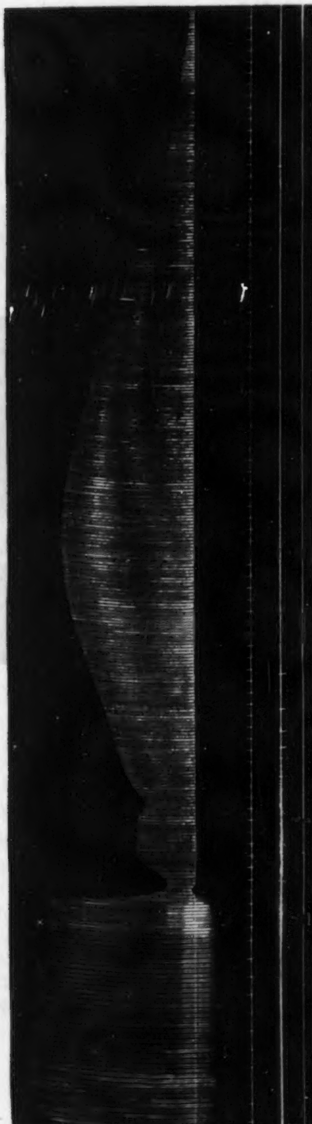

Fig. 1. In this and the following records the upper curve is that of muscular contraction on a slowly revolving drum, the middle line time in 5 seconds, and the lower line the time of the injection. In this figure the rate of perfusion in drops is also recorded. *x*, Slowed drum slightly; *1*, 2 cc. of $\frac{M}{5}$ KH_2PO_4; *2*, adrenalin chloride, 0.5 cc. of a 1:1000 solution. Reduced one-half.

decreased in height. A marked increase followed, the muscle contracting to within 5 per cent of its original height. Adrenalin in this case caused an increase of 128 per cent in the height of muscular contraction. In some muscles after the administration of large, concentrated solutions of acid potassium phosphate, adrenalin neither caused vasoconstriction nor an increase in the height of muscular contraction.

Lactic acid. The results from the inactive lactic acid were not satisfactory. It produced a marked decrease in the height of contraction but adrenalin produced a betterment of only 2 to 4 per cent in some cases and in many it had no bettering effect.

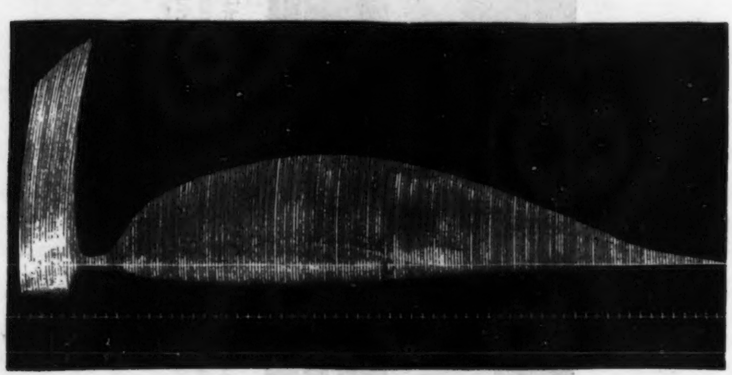

Fig. 2. *1*, 1 cc. $\frac{M}{15}$ Mallinckrodt lactic acid; *2*, epinephrin, 0.75 cc. of a 1:1000 solution. Reduced one-third.

With the use of Mallinckrodt's lactic acid good results were obtained. This acid was dextro-rotary, 6.91° at 22.5°C., which is possibly too high for pure sarcolactic acid. There appeared to be no organic matter or sugar present. In figure 2 at *1*, while the muscle was still developing its treppe 1 cc. of an $\frac{M}{15}$ solution was injected into the perfusion fluid. There resulted an immediate decrease of 95.6 per cent in the height of muscular contraction. At *2*, epinephrin (0.75 cc. of a 1: 1000 solution) was injected from which there resulted immediately a betterment, the maximum being 127 per cent.

Sarcolactic acid. In figure 3 at *1*, while the treppe was still developing 1 cc. of about an $\frac{M}{3}$ solution of sarcolactic acid was injected.[2] There resulted as in figure 2, *1*, a marked drop in the activity of the muscle of 65 per cent with a slight development of contracture. At *2*, epinephrin (1 cc. of a 1: 1000 solution) was injected into the perfusion fluid. An increase of 91.7 per cent in muscular activity was the result.

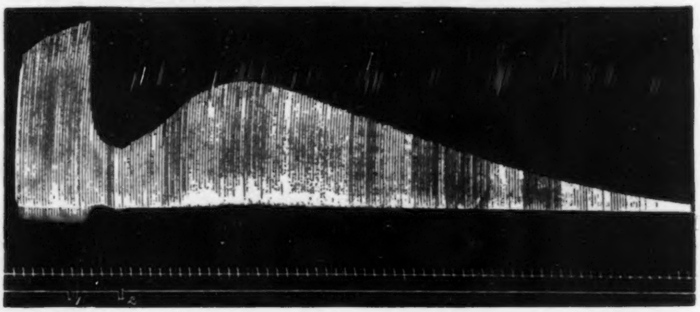

Fig. 3. *1*, sarcolactic acid $\frac{M}{3}$, 1 cc.; *2*, epinephrin, 1 cc. of a 1:1000 solution Reduced one-fifth.

SUMMARY

Adrenalin (0.5 to 1 cc. of a 1: 1000 solution) counteracts the induced fatigue produced by the perfusion of fatigue substances such as sarcolactic acid, lactic acid and acid potassium phosphate through the muscle in identically the same way as it does the fatigue produced normally in active muscles.

Adrenalin in some cases has no bettering effect. In these muscles it also has no ability to produce vasoconstriction.

[2] On account of the small quantity available the strength of the sarcolactic acid and the optical rotation could not be accurately determined. The optical rotation of this solution when made up to 25 cc. at 23°C. was 0.57° dextro-rotary. This solution of sarcolactic acid, 25 cc., was placed in a beaker and allowed to evaporate to a thick syrupy liquid which amounted to 1 cc. This was then diluted with 3 cc. of water and, if it were pure lactic acid, it would make approximately an $\frac{M}{3}$ solution.

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IX. THE ANTAGONISTIC ACTION OF EPINEPHRIN TO THE FATIGUE PRODUCED BY THE PERFUSION OF ACID SODIUM PHOSPHATE

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Burridge (1) noted that acid sodium phosphate had an action upon contracting muscles similar to that produced by the fatigue substances, acid potassium phosphate, lactic acid and carbon dioxide. The muscle showed a marked decrease in the ability to do work after being treated with this acid, the indirect response to electrical stimulation disappearing before the direct response.

Since adrenalin counteracts the fatiguing effects of sarcolactic acid, lactic acid and acid potassium phosphate, readings were made to note if adrenalin would also counteract the fatiguing action of acid sodium phosphate.

METHOD

The method was the same as that described by Gruber and Kretschmer(2). The injections of acid sodium phosphate were always made before the fatigue from excitation could set in. Both make and break shocks were used to avoid polarization and to increase the rate of stimulation.

RESULTS

The results with this acid resemble those obtained by Gruber and Kretschmer upon experimentation with sarcolactic acid, lactic acid and mono-potassium phosphate. A marked drop in the height of contraction usually resulted from the first injection (see fig. 1). Sometimes however, three or more injections were necessary to produce a marked drop in the muscle curve (see fig. 2). In figure 1 at *1*, 1 cc. of a warm $\frac{M}{5}$ acid sodium phosphate solution was injected into the perfusion

Fig. 1. In this and the following record the upper curve represents muscular contraction, the middle the time in 5 seconds and the lower the point of the injection. Make and break shock contractions. Rate of stimluation was 84 times per minute. *1*, 1 cc. of an $\frac{M}{5}$ NaH_2PO_4; *2*, 1 cc. epinephrin, 1 : 1000 solution. Reduced one-third.

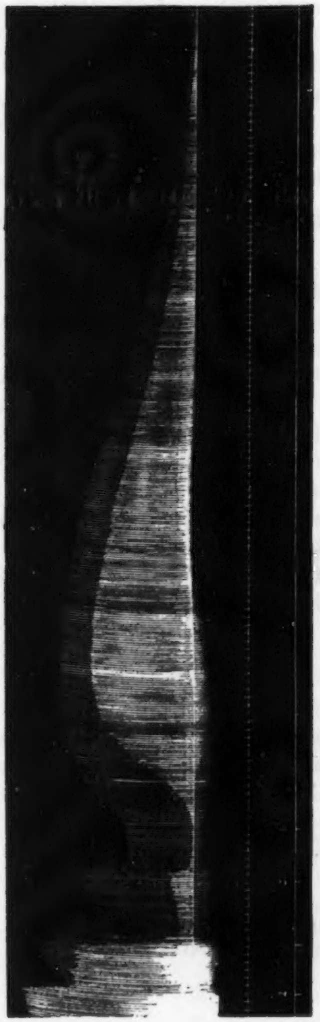

Fig. 2. *1*, 1 cc. $\frac{M}{5}$ NaH_2PO_4; *2* and *3*, 2 cc. each of $\frac{M}{5}$ NaH_2PO_4; *4*, epinephrin, 1 cc. of a 1 : 1000 solution. Reduced one-third.

fluid. There resulted a decrease of 97 per cent in the height of muscular contraction. At *2*, epinephrin (1 cc. of a 1 : 1000 solution) was injected, the rate of flow of the perfusion fluid decreased from 60 drops per minute to 1 drop per 5 minutes with an increase in the height of muscular contraction of 104.5 per cent.

In the beginning of the experiment in figure 2 the make shock was maximal but the break shock was super-maximal as shown after the injection of acid sodium phosphate. In this experiment the following injections of $\frac{M}{5}$ acid sodium phosphate solution were made at *1*, 1 cc., at *2*, 2 cc. and at *3*, 2 cc. In each case there was a recovery of the break shock but after the third injection the response to the make shock ceased. It will be noted that after each successive injection the recovery became less steep and smaller in extent. In other cases a slight recovery was noted after the third injection but following the fourth there was no recovery but a gradual decrease in the height of muscular contraction. In these muscles, in which there was this gradual loss as in the muscle from which figure 2 was made, adrenalin markedly increased the height of contraction. In figure 2 at *4*, 1 cc. of epinephrin (1: 1000 solution) was injected; the drops from the venous cannula almost ceased. The height of muscular contraction in response to the break shock increased from 15 to 31 mm. or 106 per cent. The make shocks increased from almost zero to 23 mm. in height.

SUMMARY

Epinephrin counteracts the fatigue produced by the perfusion of acid sodium phosphate as tested by the changes in the height of muscular contraction. In all cases where a marked recovery was obtained, epinephrin caused an almost complete cessation of the passage of the perfusion fluid through the muscle.

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THE INFLUENCE OF ALKALI ADMINISTRATION ON THE URINARY EXCRETION OF LACTIC ACID, AND THE POSSIBLE SIGNIFICANCE OF THE LATTER IN MAINTAINING NEUTRALITY IN THE BODY

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Quite apart from its possible relationship to the diminution of sugar in the blood and urine, the appearance of an excess of lactic acid in the blood following the injection of alkali is of interest in connection with the mechanism by which the acid-base equilibrium is maintained in the body. Because of the great frequency of acidosis as a pathologic condition, this has received much more attention than the opposite condition of alkalosis and the nature of the so-called alkaline reserves which may be called upon to combat any tendency of the H-ion to overstep the normal bounds is thought to be fairly well understood. No attention has, however been paid to the possible nature of the acid reserve available when the H-ion for any reason tends to become less than normal. Under normal conditions of life, there is a tendency to a relative increase in base when the diet contains excess of vegetable food and there may be certain clinical conditions, such as tetany, in which an alkalosis occurs. The acid-base equilibrium under these conditions is probably at first chiefly maintained by neutralization of the excess of base in the plasma by CO_2 and by conversion of NaH_2PO_4 into Na_2HPO_4. Other changes are that more ammonia is converted into urea so that the ratio between urea-N and NH_3-N in the urine falls and some of the alkali combines with protein. Besides these adjustments, the possibility exists that unoxidized acids, such as lactic acid, are used. It was more particularly to investigate this possibility that the present research was undertaken.

It might be assumed that the appearance of an excess of alkali in the organism would be entirely prevented by its combination with carbonic acid. About 0.5 gram of CO_2 are produced by a man of average weight in one minute and this would neutralize about the same amount of base as NaOH, an amount which is many times in excess of what

could possibly be absorbed from the intestines in the same period. If the neutralization of excessive base were affected solely in this way, an increase of $NaHCO_3$, and consequently of the carbonate anion HCO_3, would occur in the blood. This follows from the fact that there can be no appreciable change in the ratio of $\frac{1}{20}$ for the molecular equation $\left[\frac{H_2CO_3}{NaHCO_3,}\right]$ provided C_H remains constant. That CO_2 is used in this way (i.e., to form $NaHCO_3$) is shown in results obtained in this laboratory by R. W. Scott, who found, after injecting sodium carbonate solution, that the CO_2-content of the blood increased far beyond the amount that could be accounted for by adding the injected carbonate to the CO_2 already present in the blood. An increase in the carbonate anion has, however, been shown by the work of Hooker, Wilson and Connett (2) and R. W. Scott (3) to cause a stimulation of the respiratory center which is more or less independent of the H-ion concentration of the arterial blood, and such a stimulation of respiration certainly does not occur at any stage during alkali injection. It has recently been shown by the last mentioned worker that marked stimulation of the respiratory center may occur in decerebrate cats when there is an excess of carbonate in the blood, even when C_H of the latter is much below the normal level, indicating quite clearly that the HCO_3 anion itself must be a respiratory excitant.

To prevent accumulation of carbonate in the blood when there is excess of base, therefore, it is theoretically probable that organic acids, such as lactic, are used. We have accordingly determined the amount of this acid contained in the urine of anesthetized animals before and during the intravenous injection of an amount of alkali sufficient to lower C_H of the blood and it has been found that when the base is added in excess of the amount which could be neutralized, the salt of the organic acid accumulates in the blood and appears in the urine. Although a considerable amount of lactic acid was found to appear in the urine during the time of the observation, the quantity excreted was sufficient to account for only a small fraction of the administered alkali. This result raises the question as to whether lactic acid production would occur when the increase of alkalinity was insufficient to affect C_H of the blood appreciably, and if so, whether it could be demonstrated in the urine when excessive amounts of base were absorbed from the intestine.

METHODS

Most of the animals were anesthetized with ether, care being taken to avoid excessive struggling or dyspnea. In one or two cases urethane was used, but not as a rule since this drug may itself lead to lactic acid production. Cannulae were then inserted in the trachea, ureters, femoral artery and femoral vein. Isotonic saline solutions containing varying percentages of Na_2CO_3 were injected through the vein cannula, usually by means of Woodyatt's perfusion apparatus. Samples of about 40 cc. each of blood were removed at intervals from the artery cannula and immediately delivered into tared flasks containing 100 cc. 2 per cent HCl. The flask was then weighed and sufficient of a saturated solution of $HgCl_2$ added to precipitate the protein, the total volume being noted. After removal of the protein and mercury, an aliquot portion of the solution was saturated with $(NH_4)_2SO_4$, further acidified with H_3PO_4 and extracted, without previous evaporation, in either the Lind or the Dunbar apparatus. Our experience shows that it is advisable not to evaporate the blood filtrate prior to extracting with ether, since it is difficult to do this without some loss of lactic acid (due to anhydride formation). After extraction for 48 hours and evaporation of the ether, the residue was immediately oxidized by N/200 permanganate according to the von Fürth-Charnass method.

When urine was used, it was immediately saturated with $(NH_4)_2SO_4$ acidified and extracted with ether, the extract after removal of ether being then oxidized. Ishihara (4) states that it is advisable to precipitate the urine with phosphotungstic acid and then evaporate to small bulk before the ether extraction. Quite apart from the expense and loss of time involved in such a process, the necessity of evaporation to small bulk, even when it is done at reduced pressure, incurs the risk of loss of lactic acid. We find that prolonged ether extraction of fresh urine after saturating with ammonium sulphate removes all of the lactic acid.

We have evidence to show that the bisulphite-combining power of the oxidation distillate is a true measure of the actual lactic acid present when blood is used, but it is doubtful whether this is the case with urine. The ether extract of the latter contains a considerable amount of substance which is oxidized by permanganate so that much more of this has to be added before oxidation is complete than is the case with blood. The distillate also invariably binds a small amount of bisulphite, which is not proportional to the duration of the oxidation process.

This bisulphite-binding substance in the normal urine expressed in terms of N/20 iodine is not large. In a series of dogs' urine, it was as follows: 2.1 cc., 3.3 cc., 1.4. The variations for rabbits' urine, along with the amounts of urine used (in brackets), were: 2.4 (60 cc.); 1.5 cc. (50 cc.); 1.6 (43 cc.); 1.1 (15 cc.); 2.2 cc. (142 cc.); 2.4 (20 cc.); 1.6 cc. (55 cc.); 1.2 (30 cc.); 0.9 cc. (150 cc.); 0.9 (12 cc.). Some of the animals were fed and others starved, which accounts for the variations in the volume of urine but it is clear that the binding substance bears no proportion to the amount of urine. Although the ether was shaken with KOH and distilled after washing and drying over $CaCl_2$, it is probable that a part at least of the binding substance in most instances was derived from this source. Blanks of the reagents alone were not run with sufficient frequency but since conclusions are drawn from the results only when a very pronounced amount of bisulphite-binding substance distilled over, the omission is of no practical consequence.

In view of these facts, we must at present disregard the results on urine unless when the increase in bisulphite-combining power is very distinct, and even then we cannot be certain that lactic acid is alone, or mainly, responsible for the increase. The presumptive evidence is in favor of the oxidizable material being lactic acid, but it would be necessary, before we could be certain, to isolate and identify as the zinc salt and see whether the amount thus recovered agreed with the amount as determined by the permanganate method. We have several times attempted this isolation of zinc lactate from urines showing a large percentage of lactic acid by the oxidation method, but so far without success. Very marked qualitative reactions for lactic acid (Hopkins-Cole) are obtained in the ethereal extracts of such urines but there appears to be something present which interferes with the crystallization of the zinc lactate. Preliminary treatment of the extract with lead carbonate (to remove most of the traces of inorganic salts and of H_3PO_4 which comes over with the ether in the extraction apparatus) and subsequently with charcoal, has not been found to remove satisfactorily all of the interfering substances.

Further details of the purely biochemical aspects of our problem will be published later, but meanwhile we would point out that caution should be exercised in calculating as lactic acid all of the bisulphite-combining substance in the oxidation mixture. We note that this has been done by Dapper (5) and by Ishihara (4).

RESULTS

Lactic acid in the urine during the intravenous injection of sodium carbonate. These observations were made partly on rabbits and partly on dogs. Table 1 gives typical results on two rabbits. In experiment XVI, a total of 8.9 grams Na_2CO_3 was injected at the rate of 1 cc. per minute over a period of about four hours. There was no effect on the respiratory rate until shortly before death, although P_H rose steadily from the normal of 7.4 to 7.8. The urine was excreted in large amount and was removed in 50 to 70 cc. quantities and the lactic acid determined. A steady increase occurred, the total amount excreted during the injection being 0.650 gram. This would suffice to neutralize 4.3 per cent of the injected carbonate.

In experiment XVII, the carbonate solution was twice the strength of that used in XVI, and its injection caused complete apnea after about fifty minutes. The P_H of the blood rose to 7.9 and less urine was excreted than in the previous animal. A total of 0.245 gram lactic acid was excreted, which is sufficient to neutralize less than 2 per cent of the alkali injected.

While the results of these experiments clearly show that lactic acid or something closely related to it, appears in the urine following alkali injection, the quantity is insufficient to neutralize more than a very small fraction of the alkali.[1]

Similar results were obtained by observations on dogs (cf. table 2).

In the first of these (exp. XVIII), the alkali was injected rapidly (about 2.5 per minute) in 2 per cent solution, causing a marked rise in P_H. The urine was freely excreted and in the specimens examined there was a decided increase in lactic acid. The total amount of lactic acid was not determined so that the relationship between this and the amount of injected alkali can not be calculated.

In experiment XIX the alkaline solution was so slowly injected that there was only a slight increase in P_H. The urine was very slowly excreted and contained a total of 0.250 gram of lactic acid or about 5 per cent of the amount required to neutralize the injected alkali. This animal was anesthetized with urethane and is therefore not strictly comparable with the others, in which ether was used.

[1] Allowance was not made in calculating the amounts for the bisulphite-binding material in the normal urine and reagents. This is however so small compared with the amounts found after alkali that allowance for it would not materially affect the calculations.

TABLE 1

TIME	RESPIRATORY RATE PER MINUTE	TEMPERATURE (RECTUM)	AMOUNT OF Na_2CO_3 SOLUTION INJECTED	URINE			P_H OF BLOOD
				Excreted	Lactic acid	Total amount	
Experiment XVI. Rabbit, weight 2.1 kgm.							
		deg. C.		*cc.*	*per cent*	*grams*	
Started at							
5.20	96	39.1		50	0.010	0.005	7.4
6.10	84	38.6	48 cc. 5 per cent solution	50	0.090	0.044	
7.05			40 cc. 5 per cent solution	50	0.120	0.060	7.6
7.55	80	38.0	50 cc 5 per cent solution	70	0.240	0.170	
8.38	78	37.8	40 cc. 5 per cent solution	70	0.300	0.210	7.8
9.30	56	36.8		22	0.760	0.170	
Totals.			8.9 grams Na_2CO_3 (equivalent to 15.1 grams lactic acid)			0.65	
Experiment XVII. Rabbit, weight 3.26 kgm.							
Started at							
2.27	96	38		20			7.3
3.20	60	36	50 cc. per cent solution	50	0.220	0.110	
3.55	75		Became apneic. Stopped Na_2CO_3 after 52 cc. injected for 35 minutes. Started again				7.7
4.00					0.340	0.135	
4.30	20–40	36.4	Rabbit apneic. Injection stopped				
		Dead	35 cc. 10 per cent solution	35			7.9
Totals..............			8.5 grams Na_2CO_3 (equivalent to 14.4 grams lactic acid)			0.245	

In experiment XX, even less alkali was given although the P_H of the blood rose to 7.6. Urine excretion was somewhat more rapid and a total of 0.180 gram of lactic acid appeared or about 5.3 per cent of the amount required to neutralize the injected alkali.

In experiment XXI, the same strength of alkaline solution was injected, but more rapidly, with the result that P_H of the blood remained

TABLE 2

TIME	TEMPERATURE (RECTUM)	AMOUNT OF Na_2CO_3 SOLUTION INJECTED	URINE			P_H OF BLOOD
			Excreted	Lactic acid	Total amount	
Experiment XVIII. Dog, weight 5.6 kgm. (ether)						
	deg. C.		*cc.*	*per cent*	*grams*	
	Normal 37.1					
2.48	37.6	50 cc. 2 per cent solution				
3.12		38 cc. 2 per cent solution	(2) 50	0.130		7.6
4.05	36.0	50 cc. 2 per cent solution	(3) 20	0.106		
4.10		50 cc. 2 per cent solution				
4.30			(4) 40			7.7
4.45						
4.55		50 cc. 2 per cent solution	(5) 40			
5.15		50 cc. 2 per cent solution				
5.35		50 cc. 2 per cent solution				
5.55		50 cc. 2 per cent solution				
6.00	36.0		60*			
6.20		50 cc. 2 per cent solution				
6.25–26	35.7	45 cc. 2 per cent solution		0.200		7.9
Experiment XIX. Dog, weight 9 kgm. (urethane)						
	Normal 38.5				No urine	7.45
2.20						
3.09	38.3	50 cc. 2 per cent solution				7.5
3.15			11	0.45†	0.050	
3.55	38.5	50 cc. 2 per cent solution				
4.40		50 cc. 2 per cent solution				
5.00						
6.00			19	1.04†	0.200	About 7.6
Totals.........		3 grams (equivalent to 5.1 grams lactic acid.)			0.250 grams (equal to 5 per cent of required amount)	

TABLE 2—*Continued*

TIME	TEMPERATURE (RECTUM)	AMOUNT OF Na_2CO_3 SOLUTION INJECTED	URINE			P_H OF BLOOD
			Excreted	Lactic acid	Total amount	
Experiment XX. Dog, weight 10 kgm. (ether).						
	deg. C.		*cc.*	*per cent*	*grams*	
	38.1					7.45
3.45						
4.20		50 cc. 1 per cent solution				
5.00	37.2	50 cc. 1 per cent solution				
5.05						7.55
5.10			20	0.390	0.0180	
5.50		50 cc. 1 per cent solution				
6.40		50 cc. 1 per cent solution				
6.50			20	0.495	0.100	7.6
Totals.........		2 grams (equivalent to 3.4 grams lactic acid.)			0.180 gram (equal to 53 per cent of required amount)	
Experiment XX†. Dog, weight 16.5 kgm. (ether)						
1.05						7.4
1.09‡						
1.29		50 cc. 1 per cent solution				7.4
1.50		50 cc. 1 per cent solution				7.4
2.10		50 cc. 1 per cent solution				7.4
2.31		50 cc. 1 per cent solution				7.3
2.50		50 cc. 1 per cent solution				7.2
3.12		50 cc. 1 per cent solution				7.2
3.31		50 cc. 1 per cent solution				7.3
3.54		50 cc. 1 per cent solution	300	0.130		7.4
Totals		4 grams Na_2CO_3 (equivalent to 6.8 grams lactic acid.)			0.390 grams or nearly 6 per cent of required amount	

* Large amount between.

† In both of these the back titration was only 0.2 cc. N/20, so that there was probably more lactic acid.

‡ Ether given.

practically constant but there was marked diuresis. A total of 0.390 gram lactic acid was excreted, corresponding to about 6 per cent of the amount of alkali injected.

In the experiments of the immediately preceding group, the alkali was continuously injected while the urine was being collected. It was thought possible that a larger amount of lactic acid in proportion to the alkali injected might be obtained if the alkali were given within a short period of time at the beginning of the observation and the urine collected for a subsequent period of several hours. The results of two such observations are given in table 3, and from them it appears that contrary to expectation, relatively less of the lactic acid used to neutralize the alkali was excreted in the urine during the observation than when the alkali injections were continuous. In both experiments a marked increase in the percentage of lactic acid was observed in blood which was removed several hours after discontinuing the injections of alkali, and corresponding to this there was a continued excessive amount of lactic acid in the urine. Indeed in one of the experiments the urinary excretion of lactic acid progressively became greater. Had the observation been continued, it is evident that a much larger total amount of acid would have been recovered.

The effect produced by the oral administration of alkali on the lactic acid of the urine. Although the lactic acid excreted in the urine following intravenous injection of alkali is sufficient to neutralize only a small fraction of the administered alkali, the amount thus excreted is so much in excess of that which is apparently present in normal urine that it seemed advisable to see whether any increase would occur when alkali was given by way of the stomach. Since it is uncertain, as has already been pointed out, whether all of the bisulphite-combining material which comes over in the distillate during the oxidation of the ether extract of urine is really lactic acid, one cannot draw conclusions from the results unless decided increases are found to occur. Even in this case we cannot be certain that it is really to lactic acid that the increase is due. Notwithstanding these criticisms, we have thought it advisable to report in condensed form the results which have been obtained.

1. Two observations were conducted on a cat kept on a constant diet of fish and also fed by stomach tube at 9 a.m. daily with a soup made of minced liver, flour and water. On certain days sodium carbonate or bicarbonate was added to the soup. Any effect which the excitement and occasional struggling occasioned by passing the stomach tube might have on lactic acid excretion was largely discounted by the

TABLE 3

TIME	INJECTIONS	URINE: Excreted	URINE: Lactic acid, per cent	URINE: Lactic acid, total amount	BLOOD: P_H	BLOOD: Lactic acid, per cent	NOTES
	Experiment XXII.	***Weight 13.2 kgm. (ether)***					
		cc.		*grams*			
9.40		50.0	0.034	0.0169			Bladder urine removed and cannulae inserted
10.10	4 grams Na_2CO_3 in						
10.55	200 c. 0.9 per cent NaCl						
10.56		116.0	0.057	0.066			Urine alkaline (litmus)
11.20					7.4		Urine slower
							Blood pressure 130
							Temperature 37.8
12.35	5 grams urea injected						Urea injected for diuresis
1.00		36.0	0.130	0.047			
2.45		50.0	0.210	0.105			
2.50						0.187	

Total amount of lactic acid (after subtracting normal), 0.164 gram.

Total alkali injected, 4 grams Na_2CO_3, requiring 6.79 grams lactic acid to neutralize.

The lactic acid excreted sufficed to neutralize 2.1 per cent of administered alkali.

TIME	INJECTIONS	URINE: Excreted	URINE: Lactic acid, per cent	URINE: Lactic acid, total amount	BLOOD: P_H	BLOOD: Lactic acid, per cent	NOTES
	Experiment XXV.	***Weight 13.2 kgm. (ether)***					
9.20		80.0					
9.48	2.65 grams Na_2CO_3						No change in blood
10.17	injected						pressure or respiration by injection
10.18							Urine alkaline (litmus)
10.23						0.054	
11.00	5 grams urea injected						Urea for diuresis
12.00		41.5	0.117	0.048			
1.30	5 grams urea injected						Urea for diuresis
3.00	2.5 grams urea injected						Blood pressure 110 mm. Hg.
							Temperature 38°C.
4.10		51.0	0.094	0.048			
4.12						0.126	

Total amount of lactic acid, 0.096 gram.

Total alkali injected, 2.65 gram Na_2CO_3, requiring 4.5 grams lactic acid to neutralize.

The lactic acid excreted sufficed to neutralize 2.1 per cent of the administered alkali.

procedure being gone through whether or not the soup contained the alkali. Three hours after giving the soup (at 12 noon), the bladder was emptied by catheter and after measuring the specimen of urine so obtained, portions were taken for determination of P_H and ammonia, the remainder being added to the urine which had collected during the previous twenty-four hours in a thoroughly clean metabolism cage. The lactic acid and nitrogen were then determined in the total 24-hour specimen. The cat was fed 100 gram fish immediately after the urine had been collected.

Table 4 gives the result of the two experiments. In cat I, after a preliminary period of eight days during which no alkali was given, there followed one of six days during which 0.5 gram Na_2CO_3 per kg. body weight was mixed with the food daily. This was followed by a third period without alkali.

The carbonate was retained by the stomach except on the second day after the start, when most of the soup was vomited. That the alkali was absorbed and had called into play the neutralizing mechanisms of the body, is indicated by the increase in P_H of the urine and by diminution in the ammonia excretion.

Regarding lactic acid it will be noted that the daily excretion was fairly steady, with the exception of one day, until the alkali period when it rose from an average of about 0.007 gram to 0.012 gram on the first and 0.022 gram on the third days, after which, although the alkali was still being given, it fell back to the normal level. A slight degree of diuresis resulted from the alkali administration, but that this diuresis alone was apparently not responsible for the increase in lactic acid is evidenced by the fact that the actual amount of bisulphite-combining substance in the distillate was increased from a steady average of about 1.7 cc. N/20 for the normal urines to over 4 and 5 cc. on the first and third alkali days, respectively. The increase in supposed lactic acid is, however, very small and the result must be considered as quite inconclusive.

It is interesting to note that on one day in the preliminary normal period (July 24) the lactic acid rose very decidedly and the relative ammonia excretion fell without change in P_H. We have no explanation to offer for this deviation from the otherwise fairly constant daily values.

In the second experiment the procedure was much the same as in the first with the differences that the stomach tube was passed twice daily and the alkali was $NaHCO_3$ instead of Na_2CO_3. It was thought possible that by more prolonged absorption of the alkali from the intestine, more striking results might be secured.

TABLE 4

DAY	FOOD, ETC.	LACTIC ACID PER DIEM	URINE	TOTAL NITROGEN	PER CENT OF NITROGEN AS NH_3-N	REACTION P_H
		Cat I				
		grams	*cc.*	*gm.*		
July 18	50 cc.	0.004	43			
July 19	50 cc.	0.006	56	2.24	4.126	6.1–6.3
July 20	50 cc.	0.006	75	2.27	4.626	6.1–6.3
July 21	50 cc.	0.007	55	1.78	4.118	6.1–6.3
July 23	50 cc.	0.005	80	2.71	4.042	6.1–6.3
July 24	50 cc.	0.020	95	2.50	1.862	6.1–6.3
July 25	50 cc.	0.006	56	2.07	3.661	6.3–6.5
July 26	50 cc.	0.007	90	1.60	5.621	6.3–6.5
July 27	50 cc. + Na_2CO_3	0.012	76	1.85	2.455	8.3+
July 28	50 cc. + Na_2CO_3	Cat vomited				
July 30	50 cc. + Na_2CO_3	0.023	103	2.48	1.369	8.2–8.3
July 31	50 cc + Na_2CO_3	0.007	100	1.72	2.031	8.3+
August 1	50 cc. + Na_2CO_3	0.008	103	0.43	1.976	8.3
August 2	50 cc. + Na_2CO_3	0.005	78	2.47	4.437	8.3
August 3	Normal diet?	0.003	48	Cat vomited and no special determinations made		
August 4	50 cc.	0.012	107	4.3	7.412	8.13
August 6	50 cc.	0.005	80		7.412	6.3–6.5
August 7	100 cc.	0.004	98		4.321	6.3–6.5
August 9	100 cc.	0.007	106		2.919	6.3–6.5
August 10	100 cc.	0.007	70		6.401	6.3–6.5
August 11	100 cc.	0.004	54		2.975	6.3–6.5

Food by stomach, 50 cc. at time, containing 15 grams liver and 10 grams flour.
Fed at 9 a.m. Bladder specimen taken at 12, and after this cat fed in normal fashion approximately 100 grams of fish.
Weight of animal at beginning 2.4 kilos, on August 11, 2.6 kilos.
Na_2CO_3, 0.5 gram per kilo, from July 27 to August 2, inclusively.

DAY	FOOD, ETC.	LACTIC ACID PER DIEM	URINE	TOTAL NITROGEN	PER CENT OF NITROGEN AS NH_3-N	REACTION P_H
		Cat II. Catheter specimen				
August 7	100 grams	0.005	98		4.32	6.2–6.5
August 9	100 grams	0.007	106	2.65	2.91	6.2–6.5
August 10	100 grams	0.007	70	2.94	6.41	6.3–6.5
August 11	100 grams	0.004	54	2.80	2.97	6.3–6.5
August 14	100 grams plus 2 grams $NaHCO_3$ per kilo	0.014	116	2.90	0.64	8.1–8.3
August 15	100 grams plus 5 grams $NaHCO_3$ per kilo				2.00	8.1–8.3

TABLE 4—*Continued*

DAY	FOOD, ETC.	LACTIC ACID PER DIEM	URINE	TOTAL NITROGEN	PER CENT OF NITROGEN AS NH_3-N	REACTION P_H
Cat II. Catheter specimen—Continued						
August 16	100 grams plus 5 grams $NaHCO_3$ per kilo	0.004?	62	0.60	0.65	8.3+
August 17*	100 grams plus 5 grams $NaHCO_3$ per kilo	0.050	191	3.80	0.16	8.3+
August 18	100 grams. No alkali	0.007	200	5.80	1.67	7.5–7.7
August 19	100 grams.	0.005	90	2.34	4.73	6.3–6.5

100 gm. or cc. contained 20 gm. of meat and 10 gm. of bread. This was fed by stomach tube in two portions of 50 cc. each, one at 9 a.m., the other at 3 p.m. In the late afternoon the cat was allowed to eat in the normal fashion approximately 100 gm. of fish.

* Cat vomited once on this day.

The P_H of the urine and the relative ammonia excretion again both indicated absorption of the alkali, and the lactic acid excretion on the four days preceding those on which alkali was given averaged 0.006 gram and did not vary greatly. During the first few alkali days there was apparently only a moderate increase in lactic acid (unfortunately the estimation for the second day was lost and there is doubt as to the accuracy of that of the third), but the increase became very pronounced on the fourth, which was also the last day of the period. Diuresis was marked on this day, but it was also present on the succeeding day when, however, the lactic acid had returned to the normal level. This increase was independent of the diuresis, as was shown in the bisulphite-titration figures, which stood steadily at about 1.6 cc. N/20 solution, except in the urine of August 17, when they rose to 9.6 cc. N/20 solution. Unfortunately the cat vomited on this day and although none of the vomit appeared to mix with the urine, yet some may have done so.

An excessive excretion of nitrogen occurred on the last alkali day and on that immediately succeeding.

The results indicate that a substance which is either lactic acid, or something closely related to it chemically, tends to be excreted in greater amount in the urine when alkali is being absorbed from the intestine in sufficient quantity to change the H-ion concentration of this fluid and to lower the relative ammonia excretion. Although the amount of this substance in normal urine is tolerably constant from day to day, yet

it does occasionally become very decidedly greater independently of giving alkali (i.e., results of July 24). It is significant however that on the day on which this increase was observed to occur there was evidence of a disturbance in the acid base equilibrium, namely, a fall in the relative ammonia excretion.

OBSERVATIONS ON MAN

These were made on three healthy men living on an average summer diet of approximately the same composition from day to day but without any precise measurement of the actual amounts of its constituents. Care was however exercised to take no irregular amounts either of animal food-stuffs or of fruits and vegetables, since these might disturb the acid base equilibrium. The urine was collected for 24-hour periods with the usual precautions and total nitrogen, ammonia, or total acidity and P_H were determined, as well as lactic acid. After a certain period on the standard diet, sodium bicarbonate was given on several succeeding days and finally the normal diet alone was taken.

The results are shown in table 5. In the observations on K. and Sh., the lactic acid excretion on the normal days varied between 0.014 and 0.029 gram per diem, the amount being independent of the volume of urine excreted. In the case of St., however, the excretion was much greater, viz., between 0.05 and 0.08 gram per diem. We are inclined to believe that the greater values for St. are in part due to more thorough ether extraction. Although this difference in technic occurred between the urines of St. and those of K. and Sh., the extraction was constant for the urines of each experiment so that the results are comparable for any given individual. The more thorough extraction of the urine of St. we attribute to the use of the Dunbar extractor in place of that of Lind.

The amount of lactic acid in normal human urine was found by Ishihari (4) to be about 0.08 gram per liter. Our figures, it will be seen, are somewhat lower. Dapper (5) in a series of pathological urines found daily amounts of lactic acid varying between 0.0516 gram and 0.489 gram, the latter result being obtained in the case of mechanical liver obstruction. Most of the results lay between 0.05 gram and 0.150 gram.

With regard to the influence of alkali administration, it will be observed that the lactic acid excretion was approximately doubled in the urine of K., the increase lasting for at least two days after the alkali administration had ceased. In the case of Sh., the increase was still

TABLE 5

DAY	FOOD, ETC.	URINE	LACTIC ACID, PER DIEM	TOTAL NITROGEN	PER CENT OF NITROGEN AS NH_3N	REACTION P_H
	Subject: K.					
1	Normal diet	920	0.025	10.67	6.68	6.7–6.9
2	Normal diet	930	0.016	10.78	4.96	6.8–6.5
3	Normal diet	1030	0.014	12.33	3.77	6.5–6.7
4	Normal diet	960		10.25	4.28	6.3–6.5
5	$NaHCO_3$, 15 grams per diem	1260	0.039	8.65	1.51	8.1–8.3
6	$NaHCO_3$, 20 grams per diem	1760	0.018	11.59	0.97	8.3+
7	$NaHCO_3$, 25 grams per diem	1300*	0.038	10.69	0.41	8.3+
8	Normal diet	1450	0.033	8.33	5.136	7.1–7.3
9	Normal diet	1070	0.043	11.25	6.08	6.5–6.7
	Subject: Sh.					
1	Normal diet	935	0.029	12.72	4.53	5.5–5.7
2	Normal diet	850	0.023	10.30	3.77	5.5–5.7
3	Normal diet	1100		9.4	5.44	5.5–5.7
4	Normal diet	1250		9.6	5.47	5.5–5.7
5	Normal diet	1100	0.018	11.07	4.06	5.5–5.7
6	$NaHCO_3$, 15 grams per diem	1300	0.022	7.56	2.08	8.1–8.3
7	$NaHCO_3$, 15 grams per diem	1800	0.045	8.07	0.98	8.3+
8	$NaHCO_3$, 15 grams per diem	2300	0.092	8.00	0.83	8.3+
9	Normal diet	1100	0.048	8.10	4.16	5.5–5.7
10	Normal diet	1250	0.093	9.63	5.32	5.5–5.7
	Subject: St.					
					ACIDITY CC. N/10 NaOH REQUIRED TO NEUTRALIZE TOTAL URINE *†	
1	Normal diet	1300	0.068	14.9	247.0	6.1
2	Normal diet	1065	0.080	10.4	303.0	5.9–6.1
3	Normal diet	1050	0.050	8.7	236.2	5.9–6.1
4	Normal diet plus 15 grams $NaHCO_3$	1275	0.092	11.7	92.0	8.0+
5	Normal diet	1210	0.234	13.6	133.1	7.3
6	Normal diet	1315	0.051	17.4	190.7	6.7–6.9
7	Normal diet	1050	0.070	9.2	393.7	6.1–6.3
8	Normal diet	950	0.152	9.12	479.5	5.9–6.1
9	Normal diet	1200	0.103	8.08	462.0	5.8–6.1

* Severe diarrhea.

† The urine was saturated with neutral sodium oxalate and then titrated with N/10 NaOH to the neutral point with phenolphthalein.

more marked, rising from a normal average of 0.025 to 0.091 on the third alkali day; and again the increase continued for at least two days after discontinuance of the alkali.

The observation on St. was conducted in a somewhat different manner from those on K. and Sh. in that the alkali (15 grams $NaHCO_3$) was given on one day only, the urine being collected daily for one week following. On the alkali day a slight increase in the lactic acid excretion occurred and on the following a very decided increase. The excretion then fell to the normal for this individual for two days but rose unaccountably on the next day, when it was accompanied by an unusually high titrable acidity but by no change in P_H of the urine.

In all three observations of this group it will be observed that the H-ion concentration of the urine, the ammonia excretion and the titrable acidity indicate that on the alkali days a sufficient amount of alkali had been absorbed to affect the acid-base equilibrium of the organism.

Taking the results as a whole, the conclusion which we may draw is much the same as that in the case of the observations on cats, namely, that an ether-soluble substance, behaving like lactic acid, becomes decidedly increased in amount in the urine either during or following the administration by mouth of sufficient alkali to disturb the acid-base equilibrium of the body.

OBSERVATIONS ON STARVED AND FED RABBITS

These were undertaken in order to see whether the very decided differences which must exist in the mechanism of acid-base equilibrium in well-fed as compared with starved herbivorous animals would cause any change in the excretion of lactic acid by the urine. Three animals were observed. For a period of five days two of them (rabbits I and II) were starved (water being placed in the cages), and the third fed with abundance of carrots. For a further period of four days the previously starved rabbits (I and II) were abundantly fed. The urine was allowed to collect in bottles containing phosphoric acid placed under the metabolism cages. The urine that collected in twenty-four hours was then saturated with ammonium sulphate, extracted with ether, and lactic acid determined by the usual method. Since the animals were not catheterized, the daily quantities of urine are of course irregular, but it was thought that if the food had any influence on the lactic acid excretion it would be quite evident without such precautions. It was observed that a very large amount of permanganate had to be added to

the ether extracts before any permanent pink tinge developed in the oxidation mixture. The amount of bisulphite which combined with the distillate was by no means proportional to the amount of permanganate used, showing clearly that the ethereal extract of rabbits' urine contains a very large quantity of oxidizable material which is not lactic acid.

The amounts of bisulphite-combining material formed, expressed in terms of N/20 solution, were fairly constant for the urines of starved rabbits. This is shown by examination of the following figures, in which the cubic centimeter of N/20 bisulphite combined is given along with the cubic centimeter of urine extracted (in brackets).

1.6 (43 cc.), 1.1 (15 cc.), 2.4 (20 cc.), 1.6 (55 cc.), 1.2 (30 cc.), 0.9 (65 cc.), 0.9 (12 cc.), 0.6 (6 cc.).

TABLE 6

DAY	RABBIT I			RABBIT II			RABBIT III		
	Diet	Urine	Lactic acid	Diet	Urine	Lactic acid	Diet	Urine	Lactic acid
		cc.	mg. grams		cc.	mg. grams		cc.	mg. grams
2	Starved	43	3.3	Starved	15	2.25	Fed	285	9.0
3	Starved	20	4.9	Starved	55	3.26	Fed	290	22.0
4	Starved	30	2.45	Starved	65	1.9	Fed	370	4.5
5	Starved	13	1.85	Starved	6	1.23	Fed	152	1.0
8	Fed	185	10.75	Fed	150	13.1			
9	Fed			Fed	100	6.14			
10	Fed	150	15.54	Fed	85	6.14			
				Fed	95	8.20			

For the fed rabbits, the figures were:

2.2 (150 cc.), 5.1 (150 cc.), 0.9 (150 cc.), 0.5 (152 cc.), 4.0 (150 cc.), 6.4 (156 cc.), 3.0 (100 cc.), 7.6 (150 cc.), 3.0 (85 cc.).

Some of this material may have been derived from the reagents, but certainly not more than 1 cc. N/20 solution, so that the larger amounts distilling over in the case of the urines from fed rabbits must evidently have been derived from something in the urine itself. It is doubtful, however, whether this is lactic acid. In any case the high titrations were always obtained in urines which had been excreted in large volume, although sometimes the volume might be large without any excess of bisulphite-combining material. The results of the experiments, calculated in milligrams of lactic acid excreted in the 24-hour collections of urine, are given in table 6.

In the cases of rabbits I and II there is clearly an increased excretion of what is presumedly lactic acid on the days of liberal feeding as compared with those of starvation. In rabbit III the results are less evident.[2]

In order to eliminate the ambiguity occasioned in these results on account of the great variations in the amounts of urine excreted, another series of observations was undertaken in which the rabbits were all fed an abundance of green food (cabbage) and alkali (bicarbonate) was given on certain days (by sprinkling bicarbonate solution over it). Although the rabbits partook of the alkaline food and the urine volumes were approximately constant from day to day, there was no demonstrable difference in the amount of lactic acid excreted.

CONCLUSIONS

Injection of alkali causes lactic acid, as measured by the von Fürth-Charnass method, to appear in considerable quantity in the urine, and this may occur without any perceptible change in the hydrogen-ion concentration of the blood.

The quantity of lactic acid recoverable during the period of alkali injection is insufficient to account for more than 5 or 6 per cent of the administered alkali.

When the alkali is given in one dose and the urine collected for some hours afterward, the proportion of lactic acid in comparison with the alkali administered is no greater than during continuous alkali administration. The excretion of lactic acid under these conditions is, however, maintained at a high level.

In only a few of the experiments was any decided diminution in respiratory rate observed even when the amounts of alkali injected were sufficient to lower the hydrogen-ion concentration of the blood and urine to a marked degree.

Alkali given by stomach in sufficient amounts to cause a disturbance in the acid-base equilibrium of the body as evidenced by changes in P_H, in the NH_3 excretion and in the titrable acidity of the urine, causes a slight increase in what is presumably lactic acid in the latter fluid. This result was obtained in observations made on two cats and on three men.

[2] We are indebted to M. E. Fulk for assistance in several of the experiments on rabbits.

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VARIATIONS IN IRRITABILITY OF THE REFLEX ARC

IV. Variations in Flexion and Crossed-Extension Thresholds in Experimental Traumatic Shock, with Coincident Blood Pressure Changes

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Diminished reflexes, torpor and lessened sensibility to pain have for long been included as symptoms in typical cases of shock.[1] Just what part the lessened activity of the nervous system plays in producing the completed picture of shock is not yet clear. That it is coincident with other symptoms which are of serious import is beyond doubt. Sir Astley Cooper, quoted by Mansell-Moulin,[2] noted that the moment of transition from intense agony to perfect ease had been known to prove fatal. That diminished activity of the nervous system can be produced in experimental shock is well known to all investigators in this field. Janeway and Ewing,[3] with dogs, are able after sufficient intestinal manipulation to dispense with the anaesthetic. Meltzer[4] reports loss of skin sensitivity in rabbits when the intestines are handled outside the body cavity. Henderson, Prince, and Haggard,[5] produce shock by intestinal manipulation, and as one of the criteria of its presence cite "lessened reflex activity." The phenomenon is not confined to mammals. In Goltz's[6] classical "Klopfversuch," after the frog's abdomen has been struck strongly and repeatedly, the animal passes into a "seemingly dead, stupefied condition." Recovery promptly ensues on cessation of the beating.

[1] Compare Fischer's oft-quoted description: Sammlung Klinischer Vorträge, 10 (Chirurgie, 5) 1870, 69.

[2] Mansell-Moullin: International encyclopedia of surgery, N. Y.,1 881, i, 357.

[3] Janeway and Ewing: Ann. Surg., 1914, lix, 158.

[4] Meltzer: Arch. Int. Med., 1908, i, 571.

[5] Henderson, Prince and Haggard: Journ. Amer. Med. Assoc., 1917, lxix, 965.

[6] Goltz: Arch. f. Anat. u. Phys., 1863, xxvi, 1.

Interest in shock at present is largely centered in the redistribution of blood,[7] with all the disastrous consequences of that phenomenon. This feature is doubtless most in immediate need of comprehension and control. It should not be forgotten, however, that serious as the shock effects resulting from a loss of blood from the circulation may be, there exists also a group of primary shock symptoms which "may come on so soon after injury as to be accounted for only as the result of nervous action."[8] These symptoms have been described only from observation on the human patient and on animals with the central nervous system intact, so far as I am aware. In this paper I shall report observations on some primary shock phenomena, as they appear in an animal deprived of the brain. The investigation followed two lines: *a*, quantitative measurement of the increase in thresholds of the flexion and crossed-extension reflexes, which results from the trauma of intestinal manipulation; and *b*, a study of the coincident blood pressure changes and their relation to the high reflex thresholds. The cat was used, made spinal and prepared for a study of the reflexes by methods described in a previous paper.[9] Often, in addition to pithing the brain, the cord has been either pithed or transected, usually not lower than the first dorsal vertebra, sometimes as low as the sixth thoracic. In neither case are the sensory pathways from abdomen to cord interfered with.[10] This was done to eliminate the scratch reflex. In some spinal cats this reflex is very troublesome and if not prevented renders study of the flexion and crossed-extension reflexes impossible. The most certain preventive measure I have found has been to pith a greater or less extent of the cord or to transect it. This operates, presumably, by cutting the reflex arc between the "receptive field"[11] and the hind-legs. The flexion reflex was obtained from the tibialis anticus muscle by stimulation of the posterior tibial nerve with single break induction shocks. Crossed-extension was elicited by stimulation of the same nerve in the same way. The modified Lucas liquid electrode, described

[7] Cannon, Cowell, Fraser and Hooper: Report no. 2, Special Investigation Committee, Medical Research Committee, (Great Britain). Journ. Amer. Med. Assoc., 1918, lxx, 520 and 607.

[8] Cannon: Journ. Amer. Med. Assoc., 1918, lxx, 611.

[9] Porter: This Journal, 1912, xxxi, 141.

[10] Quain: Elements of anatomy, New York, 1909, iii, 139.

[11] Sherrington: The integrative action of the nervous system, New Haven, 1906, 46.

elsewhere,[12] was used for stimulation. The threshold for flexion was always determined from a record on the drum. The movement observed in crossed-extension varied in different experiments. Sometimes the leg was held suspended by strings and movement of the entire leg observed; or the femur was immobilized by a clamp which gripped it near the knee, or by a pin passing through a drill hole in it, and extension at knee only observed. Sometimes the patellar tendon was cut and the quadriceps group of muscles caused to write its record on the drum. The threshold for crossed-extension can seldom be determined by any method with as high degree of accuracy as that of flexion. It fatigues more easily and varies more from moment to moment.[13] The changes in threshold produced by intestinal manipulation were so gross, however, as to quite overshadow inaccuracies of threshold determination. The induction coil used was calibrated according to Martin's method,[14] and the thresholds stated in Z-units. Threshold determinations were made as often as once a minute at important points in an experiment; at other times at varying intervals as conditions demanded. The trauma instituted was the one used commonly in producing experimental shock, namely, withdrawing the intestines from the abdominal cavity and manipulating them with the fingers. Sometimes, as a severer measure, they were struck with the handle of a seeker. In many experiments a simultaneous blood pressure record was obtained by a Hürthle membrane manometer connected with the carotid artery. The reflex threshold was usually followed for half an hour or more before manipulation was begun to obtain a basis of comparison, should any change occur.

The outstanding features in the results of the investigation are as follows:

1. The flexion reflex threshold may be raised by intestinal manipulation. (Experiment of July 10, 1917, and fig. 1).

[12] Porter: This Journal, 1917, xliii, 497. This electrode I have found unfailingly satisfactory. In no experiment in the series of over thirty has there been more than momentary difficulty at the point of stimulation, and the electrode has been in place in some instances six and seven hours. I now use a glass tube 25 mm. long, 5 mm. outside diameter, 3 mm. bore, narrowed in the middle by a very short constriction which reduces the bore to 1.5 mm. Other sizes are needed for very large or very small cats. This form of tube is less likely to result in compression at the point where the nerve leaves it.

[13] Porter: Loc. cit.

[14] Martin: The measurement of induction shocks, New York, 1912.

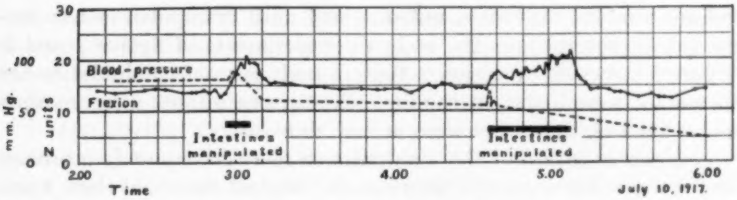

Fig. 1. Increase in the threshold stimulus for the flexion reflex in the spinal cat from manipulation of the intestines. Momentary rise in blood pressure from the same cause. Increase in threshold is apparent in the first reading after manipulation started. Recovery is prompt, eventually complete, and occurs with a lower blood pressure than before manipulation. Ordinates, units of stimulation and mm. Hg.; abscissae, time.

Experiment of July 10, 1917. Medium sized male cat. Brain pithed; cord intact

TIME	FLEXION THRESHOLD	BLOOD PRESSURE	REMARKS
	Z-units	*mm.* Hg.	
2.14	13.9	80	
2.56	13.6	80	
2.56			Manipulation begun
3.00	17.3	90	
3.04	20.6	77	
3.05			Intestines back
3.13	15.5	60	
4.36	14.3	55	
4.37		70	Manipulation begun
4.38	16.8	55	
5.08	21.6	45	
5.08			Intestines back
5.47	12.8	30	

Manipulation twice raised the threshold from a general level of about 14 Z-units to maxima of 20 and 21 Z-units. An appreciation of the amount of this increase is best obtained from the plotted curve (fig. 1). The curve shows marked and unmistakable elevations as a result of the manipulations. The total increase in threshold is six or seven times the maximum changes occurring before manipulation commenced.

2. The threshold begins to rise usually very promptly, a higher level often showing within one minute or less after manipulation has begun. This appears in figures 1, 2 and 5. The maximum threshold

values, however, are often not obtained until manipulation has proceeded for several minutes, as in the experiments of figures 1 and 2. Figure 5 shows an exception. Here in both cases of manipulation the thresholds were higher at the first reading, one minute after manipulation started, than they were at any time later.

3. Upon cessation of the manipulation there is commonly a prompt and more or less complete return to the original threshold (figs. 1 and 5). In the second half of the experiment shown in figure 1 the recovery was complete in eight minutes. It is seldom any quicker.

4. Recovery from the manipulation may be long delayed, as in the experiment of June 15, 1918 (fig. 2).

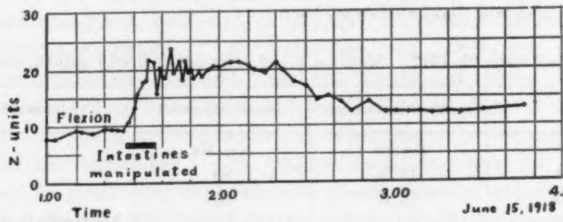

Fig. 2. Increase in the threshold stimulus for the flexion reflex in the spinal cat from manipulation of the intestines. Nearly complete recovery of threshold, but after long delay, as compared with the recovery shown in figure 1. Ordinates, units of stimulation; abscissae, time.

Experiment of June 15, 1918. Small pregnant female cat. Brain pithed; cord pithed to first dorsal vertebra

TIME	FLEXION THRESHOLD	REMARKS
	Z-units	
1.00	7.7	
1.26	9.2	
1.27		Manipulation begun
1.28	10.8	
1.37	21.3	
1.37½		Intestines back
2.10	20.9	
2.58	12.4	
3.32	12.8	

Here it was an hour and twenty minutes after the manipulation ceased before it again reached a low level, which it held steadily. It

maintained this level for fifty minutes. This is not a complete recovery, the threshold being some four units above that obtaining before manipulation. A rise of that amount in the same period of time without manipulation would not be expected.[15]

5. The attempt to raise the flexion threshold by intestinal manipulation often fails. Eleven out of twenty-four of my animals failed to show any change which could be attributed to the manipulation. In the following experiment (June 4, 1918; fig. 3), although manipulation was continued for an hour, not only was there no rise in threshold but the lowest readings of the entire experiment occurred while manipulation was in progress.

Fig. 3. Failure of the threshold stimulus of the flexion reflex in the spinal cat to be altered by intestinal manipulation lasting over an hour. Ordinates, units of stimulation; abscissae, time.

Experiment of June 4, 1918. Large male cat, much emaciated. Brain pithed; cord cut at first dorsal vertebra

TIME	FLEXION THRESHOLD	REMARKS
	Z-units	
1.24	9.9	
2.02	8.5	
2.03		Manipulation begun
2.20	8.2	
3.05		Intestines back
3.06	9.6	
3.48	10.9	

6. The rise in threshold is not due to a suddenly developed low blood pressure. Low blood pressure suggests itself naturally as a possible cause of high reflex threshold if one follows the analogy of unconsciousness from low blood pressure in syncope. But it is not the explanation here. On the contrary I have always found a rise in blood pressure at the moment intestinal manipulation begins, so that

[15] Porter: Loc. cit.

rise in blood pressure and rise in reflex threshold often coincide. This is very clearly the case in the experiment of July 10, 1917 (fig. 1). of which the protocol has already been given (p. 211). Here the rise, in millimeters of mercury, is from 80 to 90 and from 55 to 70, for the two manipulations. In other experiments, and always at the moment manipulation commenced, changes such as these have been observed: 50 to 100, 70 to 120, 30 to 60, 90 to 160, and so on. Figure 4 shows one of the blood pressure records secured during a manipulation. Blood pressure is not maintained at the new level but sinks promptly, often to a lower level, and then gradually diminishes during the remainder

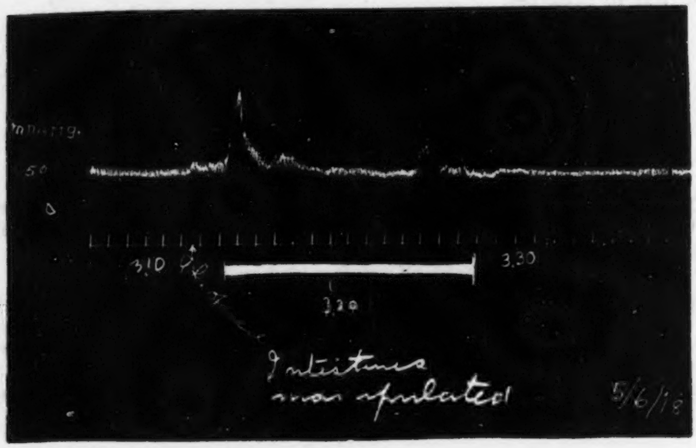

Fig. 4. Momentary rise in blood pressure (from 50 to 150 mm. Hg.) at the beginning of intestinal manipulation in the spinal cat. (Hürthle membrane manometer; calibration at left). Abdomen opened at 3.12½. Intestinal manipulation indicated by the heavy white line.

of the experiment. (See figs. 1 and 6.) When, therefore, there is a recovery of reflex threshold, it occurs with a blood pressure lower than at any previous time in the experiment. For example, in the experiment of July 10, 1917 (p. 211) at 5.47 the reflex threshold was 12.8 and blood pressure 30 mm. Hg. When the experiment started, at 2.14, reflex threshold was 13.9 and blood pressure 80 mm. Hg. Clearly general blood pressure is not connected with the reflex changes.

7. The threshold of the crossed-extension reflex may be raised by intestinal manipulation. (Experiment of May 21, 1918 and fig. 5.)

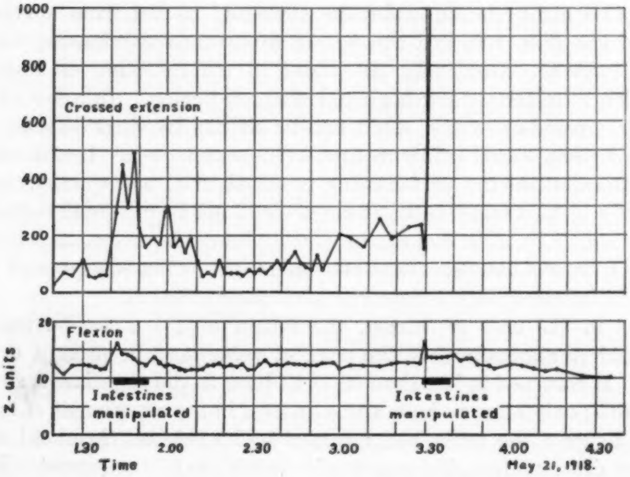

Fig. 5. Increase in the thresholds of both flexion and crossed-extension reflexes in the spinal cat following intestinal manipulation. Highest thresholds for flexion reached immediately after manipulation began (Cf. figs. 1 and 2). Disappearance of crossed-extension after the second manipulation. Ordinates, units of stimulation; abscissae, time.

Experiment of May 21, 1918. Small female cat. Brain pithed, and cord cut at first dorsal vertebra. Crossed-extension observed from movement at knee, femur held in clamp.

TIME	FLEXION THRESHOLD	CROSSED-EXTENSION THRESHOLD	REMARKS
1.20	12.4	32.9	
1.38	11.8	63.0	
1.41			Manipulation begun
1.42	16.1	283	
1.48	13.4	491	
1.52	12.6	163	
1.53			Intestines back
2.12	11.8	63	
3.28	12.8	245	
3.29			Manipulation begun
3.30	13.9	Inelicitable	
3.39			Intestines back
3.40	14.1	Inelicitable	
4.35	10.3	Inelicitable	

The first manipulation raised the threshold to 491 from a general level of less than 100, and the second threw crossed-extension out of action entirely. Obviously the effect of manipulation on crossed-extension is relatively much greater than on flexion. In a few of my animals crossed-extension could not be elicited by single shocks. In others it disappeared before manipulation commenced. In still others it disappeared during or following manipulation, but whether spontaneously or as a result of the trauma could not be told with certainty in all cases. In nine out of the sixteen cases, however, in which a study of crossed-extension was attempted, the reflex was present and could be followed both before and after manipulation.

8. As in the case of flexion, the return of the crossed-extension threshold to approximately the original value may be prompt or delayed. It returned in less than one-half hour in the experiment shown in figure 7; only after an hour and a quarter in the experiment of figure 6. In figure 5 the reflex had returned to its original threshold after the first manipulation, but was unable to withstand the second. Flexion, however, recovered from the second as well or better than from the first. Goltz[16] reports that the production of the "seemingly dead" condition in his frogs, from blows on the abdomen, succeeded better after several repetitions of the experiment on the same animal.

9. I have never found it possible to cause the complete disappearance of the flexion reflex by intestinal manipulation. It is frequently possible to do this in the case of crossed-extension with complete recovery later. (Figs. 6 and 7.) It may however, require long protracted manipulation to bring this about. In the experiment shown in figure 6 manipulation had continued for twenty minutes, and although the threshold was high it was still readable. Four minutes after manipulation had ceased crossed-extension was lost and did not reappear for twenty minutes. In a little over an hour recovery of the original threshold was complete. By disappearance of the reflex I mean that there was no response when the secondary was completely superposed over the primary, giving a stimulation of 1200 Z-units. Stronger stimulation is impractical on account of the violent and confusing flexion response which ensues. The crossed-extension threshold started in this experiment (fig. 6), with the secondary coil 5 cm. from the primary, or 20 cm. from complete superposition. The highest threshold to which I have ever forced flexion as a result of manipulation, is 40 units from an initial threshold of 12 units.

[16] Goltz: Loc. cit.

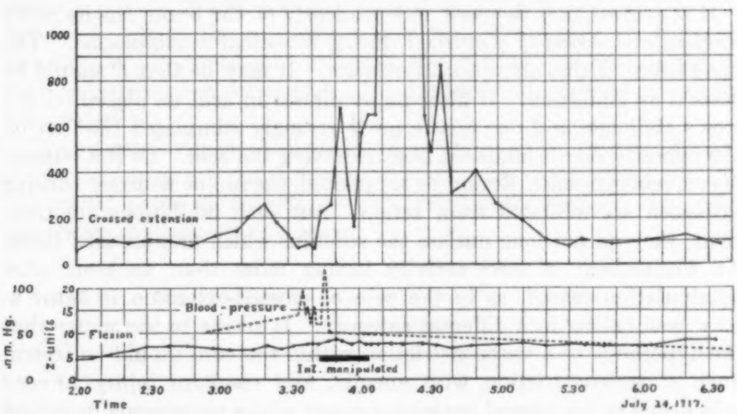

Fig. 6. Increase in threshold of the crossed-extension reflex in the spinal cat from manipulation of the intestines. No effect on flexion threshold. Complete loss of the reflex after manipulation ceased. Return after twenty minutes. Recovery of original threshold one hour later. Brief pronounced rise in blood pressure as manipulation began. Ordinates, units of stimulation and mm. Hg.; abscissae, time.

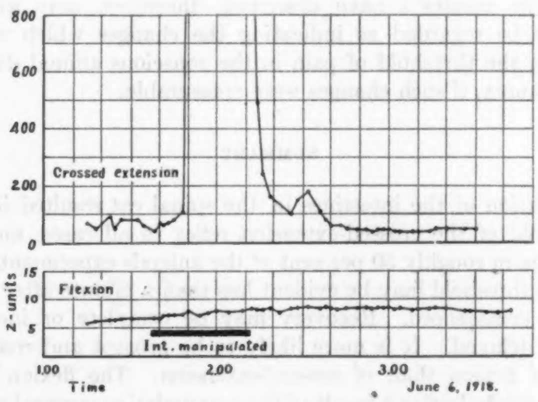

Fig. 7. Loss of crossed-extension reflex after ten minutes of intestinal manipulation. Return within three minutes after manipulation ceased and recovery of original threshold twenty-five minutes later. (Compare the slow recovery in fig. 6.) No effect on flexion threshold. Ordinates, units of stimulation; abscissae, time.

It is evident that the cord, independently of the brain, has its reflex mechanisms seriously disturbed during intestinal manipulation. The mechanism of this alteration is obscure. It may be that it should be classed as inhibition. If filter paper dipped in acid be placed on the frog's abdomen and one sciatic nerve strongly stimulated the foot on the opposite side is inhibited from removing the acid. Or if a sensory nerve causing reflex flexion be stimulated, the motor neurons causing extension are inhibited from action. But such inhibitions are transient; they do not long outlast the stimulus which first aroused them. An impairment of reflex activity lasting more than an hour after manipulation ceased, as in the case of crossed-extension in figure 6, may well belong in a different category. It seems to the writer that the hypothesis of a vasoconstriction within the cord should be tested. Such a vasoconstriction, with anemia, and resultant injury to cord cells would go far toward explaining many of the phenomena described in this paper.

Flexion and crossed-extension are elicited by nocuous stimuli, which, in the intact animal, would result in pain. When, as in the experiment of figure 1, it takes 20 Z-units to elicit the reflex during the manipulation, as compared with 13 units before manipulation, it is probable that 13 units would no longer arouse the same amount of pain in a conscious animal. The results I have described, therefore, may with some probability be regarded as indicating the changes which would be observed in the threshold of pain in the conscious animal during abdominal trauma, if such changes were measurable.

SUMMARY

Manipulation of the intestines in the spinal cat resulted in raising the threshold of the crossed-extension reflex in all cases, and of the flexion reflex in roughly 50 per cent of the animals experimented upon. Increase in threshold may be evident less than a minute after manipulation has commenced. Recovery may be complete or incomplete, prompt or delayed. It is more likely to be prompt and complete in the case of flexion than of crossed-extension. The flexion reflex is never completely lost as a result of the manipulation, crossed-extension often is. A case is described in which this did not occur until some minutes after the manipulation had ceased.

The changes in reflex threshold are not dependent on a decrease in blood pressure; on the contrary, the blood pressure rises momentarily

as manipulation starts, and this may coincide with the rise in reflex threshold. After the momentary rise the blood pressure decreases steadily, as the experiment proceeds, and the original reflex threshold may be recovered under a lower blood pressure than existed before the manipulation.

The suggestion is made, that if pain in the conscious animal could be measured during intestinal manipulation, it is probable that its threshold would show alterations roughly paralleling those of the reflex thresholds described in this paper.

THE VISCOSITY OF URINE

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DESCRIPTION OF THE VISCOSIMETER

If different fluids are permitted to traverse a capillary tube under a certain pressure, it will be found that the quantities collected within a given period of time, vary considerably. In explanation of this phenomenon it is generally stated that liquids possess a different internal friction. Thus, if distilled water and blood are employed, it will be found that the former traverses the capillary with much greater ease for the obvious reason that it contains no formed elements nor other material in suspension or solution. But inasmuch as distilled water presents the same differences when subjected to changes in temperature, it is evident that these quantitative differences are not the only cause of this phenomenon. Another factor to be considered in this connection is the viscosity of the liquid which originates in certain qualitative peculiarities of its molecular constituents. It is conceivable that the individual molecules suffer definite physical changes which lead to alterations in their adhesion and cohesion, and in turn give rise to variations in the flow of the liquid. If its relative amounts of water and solids are taken into account, a fluid is usually characterized as "thick" or "thin," while if the character of its flow alone is considered it is said to be either sticky or not sticky. Owing to the very obvious difficulties which any separation of these two factors must necessarily entail the term viscosity is usually regarded as synonymous with internal friction, i.e., it is considered as embracing the quantitative as well as the qualitative peculiarities of a liquid.

In seeking an expression for the viscosity of liquids, reference should be made to the law of Poiseuille (1) in accordance with which the outflow from a capillary tube is determined by the formula:

$$Q = \frac{d^4 h}{l}$$

Q, the quantity collected, is directly proportional to the fourth power of the diameter of the tube and to the height of the pressure, and indirectly proportional to the length of the tube. From these factors the coefficient of the viscosity may be calculated as follows:

$$Q : \frac{d^4 h}{1} = K : \frac{1^4\,1}{1} K = \frac{Q\,1}{d^4 h}$$

The viscosimeter which has been made use of by Burton-Opitz (2) in determining the viscosity of different body-fluids such as the blood, lymph and saliva, consists of a capillary tube through which the liquid to be tested is driven under a definite pressure and in a given period of time. The capillary, C, is adjusted horizontally in a receptacle filled with water. The temperature of the latter is retained at 37°C. by a flame, F, placed underneath the floor of this receptacle. The liquid to be tested is contained in tube R, the caliber of which may be varied in accordance with the quantity at hand. Its size, however, need not be large, because these determinations may be completed with less than 2 cc. of liquid. The end of tube R is connected with a pressure bottle, P, the pressure existing in this entire system being recorded by a monometer, M, upon a slowly revolving kymograph, K_1.

The free end of the capillary tube is placed against a triangular glass slide which moves transversely across its lumen. In this way the liquid escaping from it may be diverted into the weighing glass, W, directly underneath it. A sufficient quantity having been obtained, the lever, S, is moved sideways. This brings a second glass slide in front of the capillary lumen which is intended to divert the liquid into an indifferent receptacle. This arrangement enables us to begin and to cease the collection almost instantaneously.

The movements of the lever, S, are recorded upon kymograph, K_1, underneath the pressure, as well as upon a second kymograph K_2 underneath the record of a Jaquet chronograph. The latter must revolve with a speed sufficient to allow hundredths of seconds to be registered. It will be seen that these lateral deviations of the lever are communicated to an ordinary tambour, D, arranged vertically in front of the contact arm, H, and then by air transmission to tambours adjusted upon the kymographs K_1 and K_2.

This arrangement permits of the determination of the quantity of the liquid which traverses the capillary in a given period of time and under a definite pressure. In addition we know the length and the internal diameter of the capillary and need only determine, as the

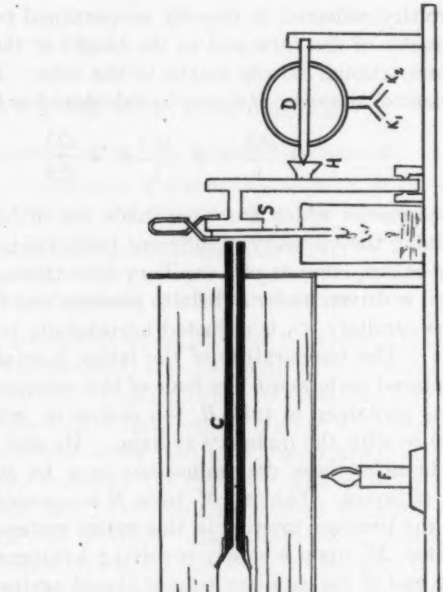

Fig. 1

sixth factor, the specific gravity of the liquid. This last determination is made with small pycnometers either before or after the calibration of the flow. The coefficient of the viscosity obtained with the help of these six factors is then compared with the coefficient of the viscosity of distilled water at 37°C. which, in accordance with the calculations of Poiseuille possesses the value of 4700. This figure is regarded as the standard, so that the viscosity of any other liquid may be expressed in terms of multiples thereof. Thus, if the coefficient of the circulating blood of the dog is 950, its viscosity is about five times as great as that of distilled water at 37°C. Consequently, the viscosity is inversely proportional to the coefficient.

THE VISCOSITY OF URINE

The values pertaining to the viscosity of normal urine are compiled in table 1. These fourteen different samples were obtained from adults upon arising in the morning. It will be seen that the coefficients vary between 3993 and 3239 and that their average value is 3823. Hence, the conclusion may be drawn that normal urine is 1.2 times as viscous as distilled water at 37°C. In this connection brief reference may also be made to the fact that this relationship in the case of the circulating blood of the dog is as 1:5.0, in the case of lymph as 1:1.5, in the case of saliva as 1:1.4 and in the case of bile as 1:1.8.

The aforesaid value corresponds to an average specific gravity of 1.0233 derived from values varying between 1.0165 and 1.0369. In view of the rather limited fluctuation of the viscosity, it seems that this factor serves as an even more reliable means of judging the character of urine than the specific gravity.

The eight determinations contained in table 2 pertain to the viscosity of the urine of normal children between the ages of four and five. In each case the sample was collected in the morning. These coefficients show values ranging between 4236 and 4029 and have given the average value of 4146. This implies that the urine of normal children of this age is only 1.1 as viscous as distilled water at 37°C. The specific gravity of these urines varies between 1.0040 and 1.0163; its average value is 1.0086.

When exposed to a lower temperature urine becomes more viscous, this change being effected more rapidly at higher temperatures than at lower. Consequently urine behaves like water and crystalloid solutions. This is clearly shown by the three experiments compiled in table 3. The differences here recorded for urine at 37° and 5°C.

TABLE 1

The viscosity of normal urine

EXPERIMENT NUMBER	SPECIFIC GRAVITY	QUANTITY	TIME	PRESSURE	COEFFICIENT OF VISCOSITY	DIFFERENCE	AVERAGE COEFFICIENT
		mgm.	*seconds*	*mm. Hg.*			
1 { 1.......	1.0228	2.5092	37.85	173.4	3836.5	156.5	3914.7
2.......	1.0228	4.3511	64.55	169.4	3993.0		
2 { 1.......	1.0369	2.9274	45.15	167.4	3833.7	91.8	3787.8
2.......	1.0369	5.0620	81.15	165.0	3741.9		
3 { 1.......	1.0238	2.9524	51.64	179.1	3268.0	58.0	3239.0
2.......	1.0238	3.3788	60.51	176.1	3210.0		
4 { 1.......	1.0285	3.4506	52.05	173.4	3814.9	4.3	3812.7
2.......	1.0285	3.0156	45.80	171.7	3810.6		
5 { 1.......	1.0165	3.3128	49.02	169.7	4020.4	54.2	3993.3
2.......	1.0165	3.5619	53.75	168.7	3966.2		
6 { 1.......	1.0169	3.5962	56.50	165.5	3881.6	86.6	3838.3
2.......	1.0169	3.3301	54.60	162.2	3795.0		
7 { 1.......	1.0223	3.0214	48.55	169.5	3686.0	58.1	3773.1
2.......	1.0223	3.4861	59.09	158.2	3744.1		
8 { 1.......	1.0277	3.2181	54.60	154.3	3814.6	7.1	3818.1
2.......	1.0277	2.9038	49.55	153.1	3821.7		
9 { 1.......	1.0185	3.0851	52.35	150.3	39.510	25.9	3938.0
2.......	1.0185	3.0852	53.30	148.6	3925.1		
10 { 1.......	1.0260	2.7280	43.45	165.7	3790.4	107.4	3844.1
2.......	1.0260	3.3000	51.80	163.5	3897.8		
11 { 1.......	1.0200	2.8335	46.05	160.2	3864.7	97.6	3913.5
2.......	1.0200	3.2444	52.08	158.2	3962.3		
12 { 1.......	1.0235	2.2481	36.42	155.1	3990.9	34.6	3973.6
2.......	1.0235	3.7512	61.85	153.8	3956.3		
13 { 1.......	1.0202	3.7429	65.27	150.5	3833.1	17.0	3841.6
2.......	1.0202	2.7440	47.80	150.0	3850.1		
14 { 1.......	1.0231	3.6758	56.41	169.4	3858.1	23.9	3846.1
2.......	1.0231	3.1653	49.52	167.2	3834.2		
Average..	1.0233						3823.8

TABLE 2

The viscosity of urine of children

EXPERIMENT NUMBER		SPECIFIC GRAVITY	QUANTITY	TIME	PRESSURE	COEFFICIENT OF VISCOSITY	DIFFERENCE	AVERAGE COEFFICIENT
			mgm.	*seconds*	*mm. Hg.*			
1	1........	1.0064	3.1416	54.80	140.6	4158.3	15.9	4150.3
	2........	1.0064	2.6453	47.62	137.4	4142.4		
2	1........	1.0040	4.1266	71.20	140.2	4226.0	16.5	4234.2
	2........	1.0040	3.0782	53.63	138.3	4242.5		
3	1........	1.0103	3.7820	66.60	138.5	4165.0	40.7	4144.6
	2........	1.0103	2.6336	47.91	135.4	4124.3		
4	1........	1.0081	2.7531	50.10	132.8	4212.6	48.4	4236.8
	2........	1.0081	2.5409	46.95	129.3	4261.0		
5	1........	1.0068	3.3268	60.70	138.5	4033.7	68.7	4068.0
	2........	1.0068	2.6418	47.81	137.3	4102.4		
6	1........	1.0098	3.3525	60.50	136.7	4120.3	19.4	4130.0
	2........	1.0098	2.7916	51.81	132.3	4139.7		
7	1........	1.0073	3.5858	64.20	136.4	4172.0	7.6	4175.8
	2........	1.0073	2.8900	52.81	133.4	4179.6		
8	1........	1.0163	3.2724	61.88	133.9	4006.5	45.6	4029.3
	2........	1.0163	1.7743	53.50	130.9	4052.1		
Average..		1.0086						4146.1

TABLE 3

The viscosity of urine at different temperatures

EXPERIMENT NUMBER		SPECIFIC GRAVITY	QUANTITY	TIME	PRESSURE	COEFFICIENT OF VISCOSITY	DIFFERENCE
			mgm.	*seconds*	*mm. Hg.*		
1	At 5°C..........	1.0242	2.0454	72.25	114.4	2479.6	1566.6
	At 20°C..........	1.0217	3.2388	73.85	153.1	2877.6	
	At 37°C..........	1.0161	4.3407	73.82	146.8	4046.2	
2	At 5°C..........	1.0304	2.5113	81.31	133.1	2312.9	1670.6
	At 20°C..........	1.0280	3.0732	72.34	150.5	2818.3	
	At 37°C..........	1.0238	3.8681	69.35	142.4	3983.5	
3	At 5°C..........	1.0324	1.7630	58.12	136.0	2217.3	1637.3
	At 20°C..........	1.0263	1.8628	57.71	134.9	2383.8	
	At 37°C..........	1.0261	3.2311	61.74	135.8	3854.6	

amount to about 1600 points, corresponding to a viscosity almost twice as great as normal. It is noted, however, that the increase between 37° and 20°C. is very much greater than that between 20° and 5°C. so that a curved line is obtained, the concavity of which is turned upwards. In perfect harmony with these changes it is observed that the specific gravity increases on cooling; the greatest difference here obtained amounts to as much as 8 points.

The effect of water drinking is clearly betrayed by the experiments contained in table 4. In each case three glasses of water were taken very shortly after the completion of the determination of the normal viscosity. A second determination was then made after an interval of from thirty to ninety minutes. While all three experiments show a very decided diminution in the viscosity of the urine, the first has

TABLE 4

The viscosity of the urine after water drinking

EXPERIMENT NUMBER		SPECIFIC GRAVITY	QUANTITY	TIME	PRESSURE	COEFFICIENT OF VISCOSITY	DIFFERENCE
			mgm.	*seconds*	*mm. Hg.*		
1	Normal	1.0224	3.9566	67.72	153.7	3816.0	314.1
	After water	1.0068	3.4914	56.92	151.4	4130.1	
2	Normal	1.0184	3.4320	61.21	143.0	3951.3	248.8
	After water	1.0083	3.5544	60.92	141.4	4200.1	
3	Normal	1.0202	3.7429	65.27	150.5	3833.1	138.9
	After water	1.0068	3.1683	55.71	146.3	3972.0	

given a difference of more than 300 points, thus establishing a relationship of 1:1.1 as against the normal relationship of 1:1.2. The specific gravity shows an equally decisive decrease from 1.0224 to 1.0068. Experiments 2 and 3 have yielded somewhat slighter differences for the reason that the second samples of urine were collected after a briefer interval of time, namely after sixty and thirty minutes respectively.

Table 5 embraces three experiments showing that the deposition of the urates increases the viscosity in a considerable measure. The greatest difference was recorded in experiment 3 and amounted to 1670 points. In other words, while the sample of normal urine was only 1.1 as viscous as distilled water at 37°C., the urates increased this value to 2.0. The specific gravity pursued in this case a course parallel to the viscosity.

Ammoniacal urine shows a tendency to become more viscous while its specific gravity remains practically unaltered (table 6), but care must be taken not to add a larger amount of ammonia than is absolutely necessary to deposit the phosphates. The greatest difference here obtained amounts to only 85 points and cannot therefore be said to be of special significance.

TABLE 5

The viscosity of urine on deposition of urates

EXPERIMENT NUMBER		SPECIFIC GRAVITY	QUANTITY	TIME	PRESSURE	COEFFICIENT OF VISCOSITY	DIFFERENCE
			mgm.	*seconds*	*mm. Hg.*		
1	Normal	1.0261	3.2311	61.74	135.8	3854.6	1637.3
	Urates	1.0324	1.7630	58.12	136.0	2217.3	
2	Normal	1.0161	4.3407	73.82	146.8	4046.2	1566.6
	Urates	1.0242	2.0454	72.25	114.4	2479.6	
3	Normal	1.0239	3.8681	68.35	142.4	3983.5	1670.6
	Urates	1.0304	2.5113	81.31	133.1	2312.9	

TABLE 6

The viscosity of ammoniacal urine (phosphates)

EXPERIMENT NUMBER		SPECIFIC GRAVITY	QUANTITY	TIME	PRESSURE	COEFFICIENT OF VISCOSITY	DIFFERENCE
			mgm.	*seconds*	*mm. Hg.*		
1	Normal	1.0261	3.2311	61.74	135.8	3854.6	16.3
	Phosphates	1.0259	3.1272	67.14	121.4	3838.3	
2	Normal	1.0235	3.6244	77.51	116.6	4021.2	73.8
	Phosphates	1.0235	3.4263	72.83	119.5	3947.4	
3	Normal	1.0224	3.9566	67.72	153.7	3816.0	85.9
	Phosphates	1.0235	2.3101	44.81	138.3	3730.1	

Table 7 contains the determinations of the viscosity and specific gravity of ten samples of albuminous urine. Two tests were made in each case; the average coefficient derived from these being given in the last column of the table. Accurate quantitative data regarding the percentage of albumin present in these urines not being available, the figures in the third column have been obtained by contrasting these

different samples with urine which on boiling, became practically solid. The latter was assumed to contain 1.0 per cent of albumin.

The average coefficient of the viscosity amounts to 3829 and the average specific gravity to 1.0197. It will be noted, however, that

TABLE 7

The viscosity of albuminous urine

EXPERIMENT NUMBER		SPECIFIC GRAVITY	PER CENT OF ALBUMIN	QUANTITY	TIME	PRESSURE	COEFFICIENT OF VISCOSITY	DIFFERENCE	AVERAGE COEFFICIENT
				mgm.	seconds	mm. Hg.			
1	1.....	1.0109	0.7	3.7589	63.30	147.4	4090.4	39.7	4110.2
	2.....	1.0109	0.7	2.8278	47.80	145.4	4130.1		
2	1.....	1.0137	0.7	3.1888	51.00	153.4	4126.4	79.1	4165.9
	2.....	1.0137	0.7	2.9171	46.20	152.0	4205.5		
3	1.....	1.0200	0.5	2.7908	47.70	149.5	3930.1	25.2	3942.7
	2.....	1.0200	0.5	3.1620	54.10	148.4	3955.3		
4	1.....	1.0164	0.6	2.7343	56.51	120.4	4057.9	37.4	4039.2
	2.....	1.0164	0.6	2.8671	61.20	118.0	4020.5		
5	1.....	1.0089	0.5	2.4728	53.40	120.4	3912.8	57.6	3941.6
	2.....	1.0089	0.5	2.4772	53.25	119.2	3970.4		
6	1.....	1.0331	1.0	2.1617	60.60	116.8	3024.6	26.9	3038.0
	2.....	1.0331	1.0	1.8162	51.24	115.4	3051.5		
7	1.....	1.0227	0.9	2.0807	46.95	115.2	3860.7	67.7	3894.5
	2.....	1.0227	0.9	1.9965	44.35	115.0	3928.4		
8	1.....	1.0219	0.9	2.1824	46.10	125.4	3823.4	57.0	3851.9
	2.....	1.0219	0.9	2.0698	43.22	124.8	3880.4		
9	1.....	1.0238	0.9	2.8931	49.97	169.5	3441.8	84.3	3483.9
	2.....	1.0238	0.9	2.7644	46.91	168.4	3526.1		
10	1.....	1.0255	0.8	3.5766	61.00	151.3	3878.8	94.3	3831.6
	2.....	1.0255	0.8	2.5454	44.70	150.6	3784.5		
Average		1019.7							3829.9

these determinations fall into two groups of five each, the first of which presents the average value of 4039 and the second the average value of 3619. The average specific gravity amounts to 1.0139 and 1.0254 respectively. This difference in the results appears to be dependent

upon the fact that the samples of urine employed for experiments 6 to 10 contain casts. Obviously, therefore, the ordinary glomerular nephritis gives rise to a urine of low viscosity because the ratio here found is as 1:1.1. The involvement of the tubules, on the other hand,

TABLE 8

The viscosity of diabetic urine

EXPERIMENT NUMBER		SPECIFIC GRAVITY	CONTENT IN SUGAR	QUANTITY	TIME	PRESSURE	COEFFICIENT OF VISCOSITY	DIFFERENCE	AVERAGE COEFFICIENT
			per cent	*mgm.*	*seconds*	*mm.* Hg.			
1	1.....	1.0072	2.5	2.3442	47.70	124.9	4009.9	94.6	4037.2
	2.....	1.0072	2.5	2.0968	42.05	123.8	4104.5		
2	1.....	1.0217	2.5	2.7776	42.15	165.3	4004.8	19.5	4014.5
	2.....	1.0217	2.5	3.2883	50.02	164.1	4024.3		
3	1.....	1.0247	2.7	3.7180	79.05	125.4	3756.8	35.8	3738.9
	2.....	1.0247	2.7	2.8376	61.80	123.6	3721.0		
4	1.....	1.0213	2.5	2.4084	44.75	134.9	4009.2	17.2	4017.8
	2.....	1.0213	2.5	2.2441	41.52	134.9	4026.4		
5	1.....	1.0302	2.7	2.1501	43.20	136.4	3635.1	14.0	3628.1
	2.....	1.0302	2.7	2.1056	43.20	134.1	3621.1		
6	1.....	1.0235	2.7	1.1568	35.65	84.8	3837.3	70.7	3872.6
	2.....	1.0235	2.7	1.3402	39.38	83.4	3908.0		
7	1.....	1.0231	2.5	1.3083	36.95	72.4	3929.2	92.1	3975.2
	2.....	1.0231	2.5	1.0517	36.90	71.3	4021.3		
8	1.....	1.0210	2.0	2.8190	45.95	158.2	3895.5	67.6	3929.3
	2.....	1.0210	2.0	2.8586	46.15	157.0	3963.1		
9	1.....	1.0389	3.2	2.0739	46.75	125.8	3483,4	53.0	3456.4
	2.....	1.0389	3.2	2.1091	48.90	124.2	3430.4		
10	1.....	1.0221	2.7	3.0693	55.20	148.9	3750.1	39.5	3730.3
	2.....	1.0221	2.7	2.8801	52.81	147.6	3710.6		
Average		1.0233							3840.0

increases the viscosity considerably so that the ratio becomes as 1:1.3. The urine used for experiment 6 was obtained from a case of eclampsia. Its specific gravity of 1.033 corresponds to the coefficient 3038. Consequently, this urine is almost 1.6 times as viscous as distilled water at

37°C. as against the normal ratio of 1: 1.2. These changes are also clearly betrayed by the specific gravity, but it appears that this factor is a much more uncertain means of determining the character of urine than the viscosity.

The ten experiments compiled in table 8 have been made with urine collected from patients suffering from diabetes mellitus. Excepting the first two cases in which a dicided polyuria was present, the coefficients of the viscosity do not differ from normal. Experiments 1 and 2, however, show that polyuria decreases the viscosity. The greatest difference here recorded amounts to about 200 points and corresponds to a ratio of 1.1 as against the normal ratio of 1.2.

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STUDIES ON EXPERIMENTAL SURGICAL SHOCK

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I. GENERAL CONSIDERATIONS OF EXPERIMENTAL SURGICAL SHOCK

In the consideration of the entire field of surgical shock from the experimental point of view, two features must be emphasized: *a*, that the condition termed shock by the surgeon is undoubtedly owing to a large number of causes; and *b*, that experimentally it is very difficult to reproduce the environment and all the phenomena which the surgeon calls shock.

The present series of studies has been made in order to give experimental data of as many conditions as possible which the surgeon might diagnose as shock. The data are based on a very large number of experiments performed since the first studies were undertaken in 1912, up to the present time. For purposes of investigation I have found it of practical value to classify the various conditions into two groups. In the first group are those cases in which the cardinal signs of the condition, such as decreased consciousness, decreased blood pressure, shallow, gasping respiration, subnormal temperature, etc., develop and supervene for some time after the exciting cause. In the second group are those cases in which a severe or fatal issue follows immediately or very closely on the action of the exciting agent.

Clinically, the first group would include most of the cases which the present-day surgeon diagnoses as shock; the chief of these would be the shock following operation. A condition presenting the clinical signs of shock and which is supposed to present a physiologico-pathologic condition similar to that in the cases in the first group, may be produced by a variety of methods. The most important of these are deep anesthesia, hemorrhage, exposure of the abdominal viscera, the use of excessive heat or cold, the isolation of large vascular areas as the partial occlusion of the vena cava, the production of cerebral anemia and the injection of drugs, peptones and oils.

It may be seen from the foregoing that but few of the methods used to produce the shock condition can be compared to the manner in which shock occurs clinically. Most of the methods aim, primarily, at a reduction of blood pressure. It is definitely known that some of them, and probably most of them, produce a decrease in the volume of circulating fluid. While the studies following these various methods of producing an experimental condition having the signs of shock have been of inestimable value in that they have added to the knowledge of the physiology of low blood pressure, it should be noted that they explain in only a limited number of cases what the possible etiologic factors in shock could be.

The second group, clinically, includes most of the classical cases of shock given in the literature of the subject. It was in reference to this type of case that the term shock was first applied, namely, when sudden death occurs or alarming symptoms develop immediately following an accident or operative procedure in which no definite cause of death is found. It is shock of this type, particularly that occurring in the operating room, that has strongly impressed the surgeon with the idea that the nervous system is essentially and primarily at fault in the production of shock.

The environment reproducing the condition included in the second group is hard to obtain experimentally. Very little experimental work has therefore been done on the investigation of sudden death associated with accidental trauma or trauma occurring in the operating room. The crushing of joints in decerebrated animals has occasionally produced death, and the sudden trauma to the whole thigh of an anesthetized animal has also been fatal.[1] In general, experimental work has not reduplicated the clinical observations in regard to the class of cases included in this group.

Practically all investigators of the shock problem have undertaken the investigation with the idea of proving or disproving some particular theory regarding it. Since the condition termed shock is probably due to a large number of factors, our researches must necessarily have to do with the many possible causes of obscure death. As further data are presented it seems that each of the theories developed to explain the condition of shock contains an element of truth, and that typical cases diagnosed as shock could be found to illustrate each theory. In the present studies no attempt has been made to correlate the data

[1] Personal communication from Dr. E. D. Brown.

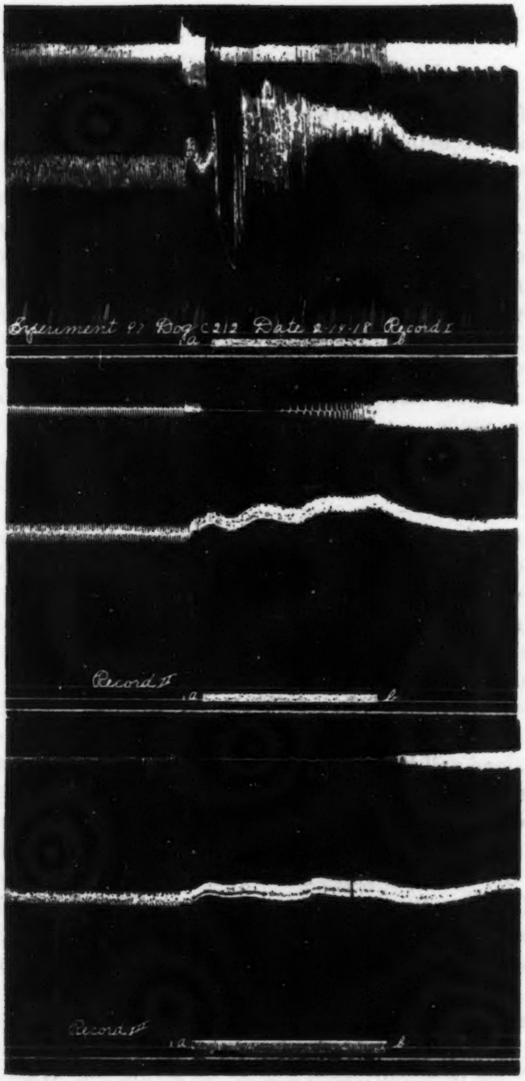

Fig. 1. Photograph of kymograph record showing the effect of different tensions of ether on the inhibitory reflex of respiration. In each record signal *A* marks the time of disconnecting the ether and signal *B* the stimulation of the right superior laryngeal nerve for two minutes. Both vagi are intact. In record 1 ether tension was 40 (uncorrected) and blood pressure was 150. Note that there was but slight inhibition of respiration. The heart was inhibited. Ether tension was gradually increased and in record 2 blood pressure was still 150 but respiration was decreased in rate and amplitude. Note the increase in the time respiration was inhibited. Record 3 shows the effect of another increase in the tension of ether. Respiration is inhibited for a much longer period.

with any of the current theories but all experiments have been devised with the view to determine some facts in regard to the many phases of the shock problem.

I am fully aware that all of these studies are not directly associated with the shock problem. However, until a comprehensive and scientific definition of shock based on known facts is made, it seems best to classify all data bearing on the causes of sudden death, the causes of low blood pressure and the phenomena of shock under the general term surgical shock. A fact that is universally true of experimental work and especially true in regard to shock should also be emphasized, namely, that direct clinical application of the experimental data should be cautiously made.

II. ETHER ANESTHESIA IN RELATION TO SURGICAL SHOCK

The anesthetic is of the greatest importance to the clinician in a consideration of post-operative shock. It is of even greater importance to the experimental investigator who would attempt to determine the causative factors in this consideraton.

All our experiments have been performed under ether anesthesia and therefore all conclusions drawn must be in regard to that anesthetic only. There were two reasons for the use of ether. First, it is the anesthetic used in the greater number of operations and therefore the results obtained will have the greatest clinical application; second, it is the anesthetic which can be most easily employed in experimental work.

In the beginning of our work on the relation of ether anesthesia to shock, we attempted to establish some definite data in regard to the activity of the various organs, nerve centers and reflexes under different tensions of ether. Boothby, by means of the Connell anesthetometer, showed that in man the anesthesia became complete at a definite tension of ether. It is reasonable to suppose that animals would react to ether in a like manner and that a standardization of ether anesthesia in reference to physiologic research would be accomplished. Two great difficulties were, however, encountered. First, the Connell anesthetometers which we personally used and as tested by the Waller gas balance, were found to have too large an error for standardization, and second, the only ether to be obtained at present is not pure enough for accurate work. These two sources of error, which we have not as yet been able to obviate, have necessitated the use of approximations only in relation to ether tensions. The errors in these approximations are

on an average probably no greater than 15 per cent. However, the data have justified several conclusions that have been of great value in our work on shock. These conclusions all have reference to ether anesthesia, as administered by an anesthetometer.

1. In general, the physiologic phenomena due to ether are remarkably constant at the same range of tensions in different dogs.

2. Under a low tension of ether, just sufficient to produce narcosis, the blood pressure remains normal for a period of at least twelve hours.

3. The various respiratory and vascular reflexes disappear at different ranges of ether tension.

4. The signs of shock can be readily produced by high tensions of ether.

It is possible for practical purposes to divide ether tensions in relation to their anesthetic action into four groups. In the first group are included the tensions that are too low to produce anesthesia. The second group includes those under which the animal is reduced to a state of surgical anesthesia but which are not high enough to depress any of the vital processes; it is impossible to kill an animal by any tension in this group, if time is not allowed to become a factor. The third group includes the tensions that produce depression of the vital processes and under which death may occur. Any tension higher than those included in this third group is incompatible with life. The range of tensions included in these various groups is approximately constant for the various animals. Of course, individual variations occur but these are usually within the limit of the error of the mechanism.

The employment of approximate ether tensions has been productive of several important findings. By the use of low tensions it can be demonstrated that an animal may be maintained in as normal a condition as it is possible to keep a narcotized animal, for many hours. This is of great value since it is thus possible to maintain a safe degree of anesthesia without its being affected by any physiologic condition of the animal or by operative procedures. It is also of great value, especially in shock experiments, to be able to administer a tension of ether which is definitely known to be too low to maintain surgical anesthesia. It is thus possible, without completely withdrawing the ether, to know when the animal has reached shock.

The respiratory and vascular reflexes undoubtedly vary under different tensions of ether but are fairly constant under the same tensions. Some reflexes are very sensitive to ether and cannot be elicited except under low tension. Other reflexes will persist as long as the involved

nerve cells functionate. In the study of any particular reflex it is of as much importance to keep the anesthetic constant as to keep the strength and rate of stimulus constant. This is impossible under most of the methods of anesthesia.

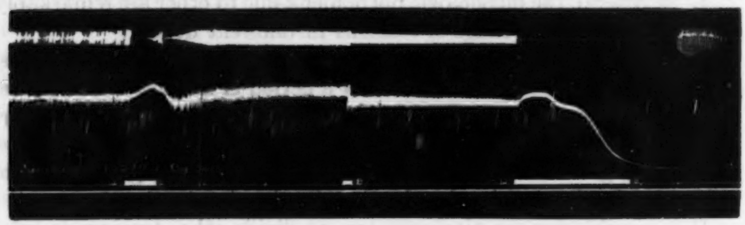

Fig. 2. Kymograph record of respiration and blood pressure. The animal at first was under a moderate ether tension (42 uncorrected). The right vagus was sectioned; the left vagus was intact. At signal *A* ether was disconnected and at signal *B* the central end of the right vagus was stimulated until respiratory movements which were inhibited at first, returned. This occupied 40 seconds. At signal *C* ether was again administered. Signal *D* marks an interval of fifteen minutes during which a high ether tension was administered (64 uncorrected). At signal *A'* ether was again disconnected and at *B'* the central end of the right vagus was stimulated for three minutes. Respiration was inhibited and after an initial rise blood pressure fell to zero.

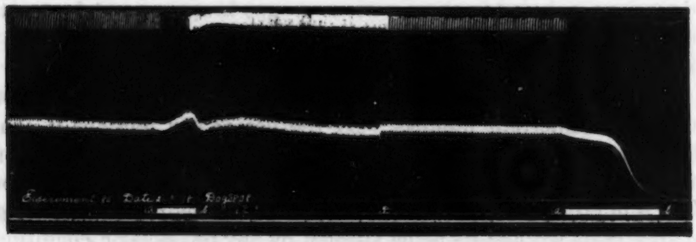

Fig. 3. Photograph of kymograph record of respiration and blood pressure. The procedure and results in this experiment were almost identical with those shown in figure 2. The minor differences are: the initial blood pressure was 120 and the uncorrected ether tension was 40; signal *D* represents a period of fourteen minutes.

A review of protocols dealing with studies of experimental shock shows that in many instances the investigator was undoubtedly studying a condition of deep etherization and not of shock. This is probably one of the most important reasons why so much of the experi-

mental data on shock is contradictory. The relation of deep etherization to the signs of shock cannot be too strongly emphasized. By the manipulations of the various tensions of ether it is possible to parallel the various stages and signs of shock. Under high tensions the blood pressure is decreased and all the other symptoms follow. However, it is quite possible to saturate an animal with ether at a tension just slightly lower than that necessary to abolish the eye reflex and to produce an obtundity of the reflexes without decreasing the blood pressure to a shock level. It is possible to obtain such results with a certainty only when the ether is administered mechanically and is not dependent in any way on the respiratory efforts of the animal.

III. REFLEX INHIBITION OF RESPIRATION AS A CAUSE OF SUDDEN DEATH DURING OPERATION

In a study of the vascular and respiratory reflexes under various tensions of ether (1) it was found that all the respiratory reflexes, except that which produces inhibition of respiratory movements, disappeared before the respiratory center failed. The excitatory respiratory reflexes disappear under a relatively high tension of ether. On the contrary, instead of a depression of the inhibitory reflex being caused by ether, a relative increase at least is quite common. Thus it was possible under deep etherization, in some instances, actually to kill an animal by prolonged stimulation of the nerve fibers that inhibit respiration. It was believed that this phenomenon might have some bearing on the shock problem; a more complete study of it was therefore made.

The idea that death could actually be produced by the action of a nerve reflex has been very prevalent with clinicians but has very little clinical or experimental evidence to support it. For this reason our experiments are important even if all the factors involved have not been determined and even if it is not possible at the present time to make a definite clinical application of the data.

The experiments were performed on dogs. The animals were etherized in a closed cabinet, intubated and the anesthesia usually maintained with a Connell (2) anesthetometer. In some experiments a modified McGrath (3) method of anesthesia was employed in order to determine if the method of administering ether were a factor in the results obtained. Respiration and carotid blood pressure were recorded (mercury, and in some experiments, membrane manometer). Ether was always discontinued during the periods of stimulation. The

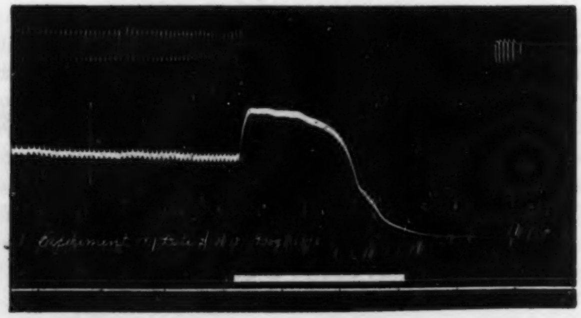

Fig. 4. Kymograph record of blood pressure and respiration. The animal was under a tension of ether which just abolished the eye reflex. Respiration was fifteen per minute and blood pressure was 105. Both vagi were sectioned. Ether was disconnected just previous to the period of stimulation. The central end of the left vagus was stimulated for two minutes and forty-five seconds. Death followed and was associated with inhibition of respiration and an initial rise in blood pressure.

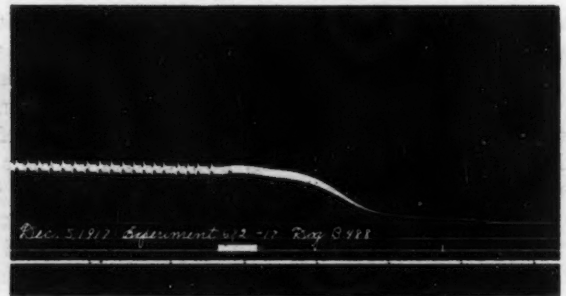

Fig. 5. Photograph of kymograph record showing sudden death following stimulation of the central end of the right vagus for thirty seconds under a high ether tension. Both vagi were sectioned. The animal had been under ether for several hours and under the same tension (58 uncorrected) for one hour. The blood pressure and respiration had been practically constant for the half hour preceding the stimulation. This record proves that death was certainly associated with an active inhibition of respiration.

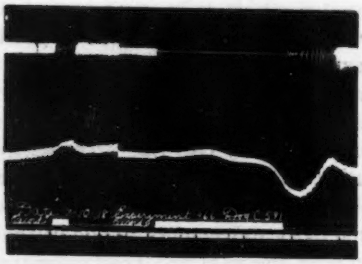

Fig. 6. Photograph of kymograph record showing recovery after a long period of inhibition. McGrath's method of anesthesia was used. The right vagus was sectioned, the left vagus was intact. Stimulation of the central end of right vagus under light ether produced partial inhibition of respiration for less than fifteen seconds (record 1). The ether tension was then increased until the eye reflex had just disappeared. Stimulation of the central end of the right vagus now inhibited respiration completely for the entire period of stimulation. When death appeared inevitable stimulation was stopped. Respiratory movements immediately occurred and the animal soon recovered (record 2). This record proves that at least in some instances death following inhibition of respiration under deep ether is an active process throughout.

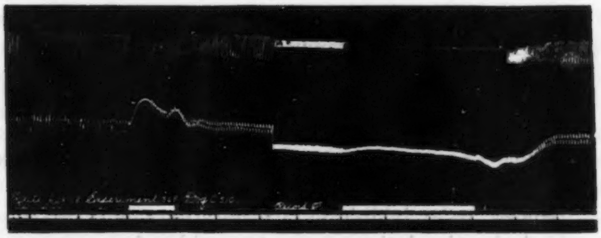

Fig. 7. Photograph of kymograph record showing increase in the inhibitory reflex of respiration under high ether tension. Both vagi were sectioned. In record 6 blood pressure was 120 and ether tension 30 (uncorrected). Stimulation of the central end of the right vagus for one minute produced inhibition of respiration for about half that period. The ether tension was then increased to 70 (uncorrected). When the eye reflex had just disappeared the vagus was again stimulated (record 7). Blood pressure had decreased to 90. The stimulation was maintained for two minutes and respiration was completely inhibited. It remained inhibited after the cessation of stimulation and blood pressure continued to fall. Insufflation was then started and in a short time recovery took place. This record shows that lack of air appears to be the important factor.

nerves were stimulated electrically in the usual manner, care being taken to keep the stimulus as near constant as possible for each experiment. In many experiments shield electrodes were used. The stimulus was always maximum in strength and rate.

From the results of the stimulation of the nerves in a large series of animals it was found that only two that were easily exposed could be relied on consistently to produce reflex inhibition of respiration. Stimulation of other nerves, such as the brachial plexus, occasionally caused a cessation of respiratory movements but only the superior laryngeal and the central ends of the vagi produced constant results. Without doubt, however, the same results may be obtained by the stimulation of other nerves when they are found to inhibit respiration, particularly those nerves containing sensory fibers supplying the upper respiratory tract.

The stimulation of either the superior laryngeal or the central end of a vagus nerve under light surgical anesthesia usually inhibits respiration for a short period. As the stimulation is continued, however, respiratory movements soon return, due either to a decrease in the reflex, or what is more probable, to an increase in the chemical stimulation of the center. It is rarely possible, under light surgical anesthesia, to inhibit respiration by the stimulation of these nerves for a long enough period to jeopardize the life of the animal. In our experiments, excluding those in which reflex inhibition of the heart was associated with the inhibition of respiration, blood pressure was never permanently lowered to a shock pressure. In a few experiments, however, there was marked depression of the blood pressure owing probably to stimulation of the depressor nerve, and complete recovery did not take place. Under ether tensions considerably less than those necessary to produce surgical narcosis, reflex inhibition of respiration might become dangerous provided the circulation was not capable of compensating for a long period of asphyxia. As the ether tension is increased the length of time that the respiratory movements are inhibited by the stimulation of those nerves is prolonged. Finally, in a large number of animals under deep etherization, the respiration fails to return; the blood pressure quickly falls and death ensues. Quite frequently death can be produced by reflex inhibition of respiration under an ether tension which will just abolish the eye reflex. A fatal result did not always occur in our experiments but the respiration, with very few exceptions, was inhibited for a much longer time under deep etherization than under light anesthesia; the opposite was rarely true.

In the earlier experiments the normal blood pressure was usually decreased as much as one-half before respiration could be inhibited long enough to produce death. However, it was found that by cautiously increasing the ether tension many animals could be killed while the blood pressure was practically normal. It was determined that such results are not dependent on the method of anesthesia or the height of blood pressure, and that they are obtained with sectioned and intact vagi.

The mechanism by means of which death is produced, associated with an inhibitory reflex of respiration under deep etherization, is not clearly

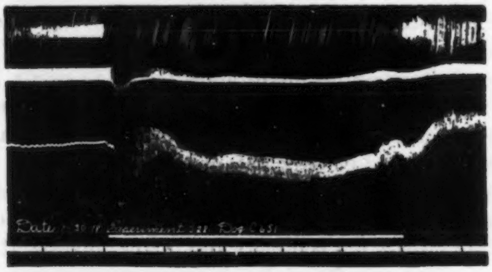

Fig. 8. Photograph of kymograph record showing a long period of inhibition of respiration associated with reflex inhibition of the heart following stimulation of the right superior laryngeal nerve under very low ether tension. The right vagus was sectioned, the left vagus was intact. Under a high ether tension such a long period of inhibition would probably have proven fatal owing to failure of the circulation. This record shows that while under very light ether tension it is rarely possible to inhibit respiration for a long period of time, a fatal result does not occur.

defined. In our experiments death occurred only when the nerves that inhibit respiration were stimulated, and it was never produced under deep etherization when respiratory movements were maintained. Death will not take place while insufflation or artificial respiration is maintained. The processes producing death seem to have been as follows:

For some unknown reason, ether does not abolish the reflexes which inhibit respiration so long as the respiratory center responds. Under deep etherization the threshold of the cells of the respiratory center is greatly increased to the chemical stimuli. A point is thus reached at which the irritability of the center is so depressed by ether that it will

not respond to the increasing amounts of carbon dioxide in the blood or to the excitatory reflexes, but will respond to the inhibitory reflexes. At this time stimulation of inhibitory fibers will produce death and, owing to the action of the high tension of ether on the circulation, a very short period of asphyxia will produce death very quickly. The ether tension under which death will follow the stimulation of the reflexes inhibiting respiration is fairly constant, although individual variations occur.

There seem to be factors other than deep etherization, although probably minor ones, involved in these experiments. The reflex producing inhibition of respiration seems very resistant to agencies which usually depress or abolish the excitatory reflexes of respiration. Thus it seems quite possible that most conditions which decrease the irritability of the respiratory center might allow death to occur by reflex inhibition of respiration providing the circulation were also depressed. The production of deep anesthesia, although probably the most common and potent, would be only one of these agencies.

The accumulation of the data substantiating the idea that depression of the respiratory center associated with a depressed circulation is the important factor is not complete and further investigation in regard to it is being carried on. The following suggestive facts, however, have been obtained: The inhibitory reflex of respiration is decreased or completely abolished during periods of hyperpnea; the period of apnea following hyperpnea under light ether anesthesia is not increased by the stimulation of the nerves which inhibit respiration, and the inhibitory reflex is decreased or abolished during the period of increased respiratory movements in the first stage of asphyxia. An animal cannot be killed by reflex inhibition of respiration during the first stage of asphyxia. However, as asphyxia is prolonged and the respiratory movements begin to decrease, stimulation of inhibitory nerves will inhibit respiration and in many experiments produce sudden death. In some experiments, while the time during which respiration is inhibited under deep ether is greatly increased over that under light ether, death cannot be produced. A slight period of asphyxia will increase the time of inhibition so that death will occur. It would seem that the lack of oxygen may be a factor. Inhibition of respiration under deep etherization frequently produces death very quickly. The blood pressure usually decreases at once and the heart soon stops beating. This result would seem to imply that death was due to or associated with other factors than asphyxia. However, a comparison

Fig. 9. Photograph of kymograph record showing a typical result following ligation of all the structures in the limbs except the major artery. Animal etherized at 8:46. Method—anesthetometer. Apparatus arranged to record carotid blood pressure, major artery to each limb exposed. Record 1 taken at 9:25, blood pressure 160. Ligatures were applied to each limb including all structures except the major artery. Record 2 taken at 9: 40, blood pressure 150. Record 3 taken at 10:40, blood pressure 114. Record 4 taken at 11:40, blood pressure 80. Animal developing the signs of shock. Ligatures removed at 11:44. Record 5 taken at 12:15, blood pressure 88.

of the curves in instances in which death was due to asphyxia under deep ether shows a close similarity to those found in the condition produced experimentally. The asphyxia under deep ether seems to explain the sudden decrease in blood pressure and stoppage of the heart, although an active process may be involved.

Under light surgical anesthesia respiration is seldom inhibited for a very long time. In a few of our experiments stimulation of the superior laryngeal nerves under an ether tension, slightly too low for surgical work, has produced a partial inhibition of respiration for as long as four to eight minutes. This period of inhibition is longer than that

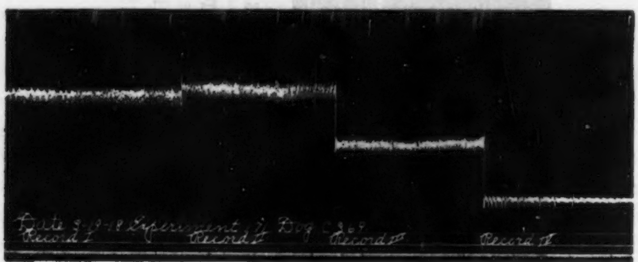

Fig. 10. Photograph of kymograph record showing a rapidly fatal termination following venous obstruction to all the limbs. Animal etherized at 8:30; method—modified McGrath. Apparatus arranged to record carotid blood pressure. Major artery to each limb exposed. Record 1 taken at 8:55, blood pressure 130. Ligatures were immediately applied to each limb including all structures except the major artery. Record 2 taken at 9:06, blood pressure 140. Record 3 taken at 10:00, blood pressure 86. Animal beginning to exhibit signs of shock. Record 4 taken at 10:30, blood pressure 34. The ligatures were now removed but the animal died during this procedure.

which produced death in several of the experiments under deep ether. Under the very low ether tension, however, blood pressure remained practically normal and death did not occur. Undoubtedly, if a high ether tension had been used, death would have followed such prolonged periods of inhibition. These results would furnish some support to the idea that the sudden death following inhibition of respiration under a high ether tension was mainly owing to a failure of the organism to compensate for the asphyxiation when saturated with a high ether tension. This relation of asphyxia to deep etherization has been previously discussed by Gatch, Gann and Mann (4). The important fact

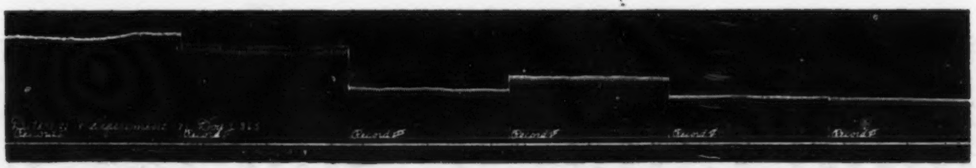

Fig. 11. Photograh of kymograph record showing slight recovery after removal of ligatures. Animal etherized at 1:19; method—modified McGrath. Apparatus arranged to record carotid blood pressure. Major artery to each limb exposed. Record 1 taken at 1:55, blood pressure 135. Ligatures were applied to each limb including all structures except the major artery immediately after taking this record. Record 2 taken at 2:05, blood pressure 120. Record 3 taken at 3:00, blood pressure 65. Animal exhibited signs of shock. The wire ligatures were removed at 3:02. Record 4 taken at 3:15, blood pressure 78. Record 5 taken at 4:00, blood pressure 50. Record 6 taken at 4:45, blood pressure 50.

Fig. 12. Photograph of record showing a sudden delayed drop in blood pressure and partial recovery after removal of the ligatures. Animal etherized at 8:30; method—anesthetometer. The apparatus was arranged to record carotid blood pressure. The major artery to each limb was exposed. Record 1 taken at 9:05, blood pressure 120. Ligatures were applied to each limb including all structures except the major artery between 9:10 to 9:15. Record 2 taken at 9:15, blood pressure 120. Record 3 taken at 10:15, blood pressure 102. Record 4 taken at 11:15, blood pressure 92. Animal beginning to exhibit signs of shock. Record 5 taken at 12:15, blood pressure 36. Ligatures were removed immediately after taking record 5. Record 6 taken at 12:40, blood pressure 60.

shown in these experiments is that the inhibitory reflex of respiration under ether anesthesia persists as long as respiratory movements occur.

Experiments dealing with the attempt to produce death by stimulating nerves other than electrically, and under conditions other than deep etherization, are not complete. However, it would seem quite possible to produce a condition of depression of the respiratory center by asphyxia, oxygen-lack or other methods, without otherwise greatly disturbing the general condition of the animal, so that the stretching of a nerve or the pulling of the mesentery might produce a serious or fatal condition owing to inhibition of respiration.[2]

What practical bearing such experiments have on the shock problem, it is impossible to state at the present time. Death is produced so suddenly and strikingly under these conditions that it seems highly probable the same effects have occurred in man.[3] It is quite possible that deep etherization and inhibition of respiration, while operations are being performed in the region of the neck, axilla and diaphragm, regions in which traumatic procedures are prone to produce stoppage of respiration, are responsible for some of the sudden deaths on the operating table which the surgeon has diagnosed as due to shock. It should be noted that the depth of anesthesia at which inhibitory reflexes become dangerous is no greater than that which some surgeons employ.

SUMMARY

Ether tensions that will decrease or abolish the excitatory reflexes of respiration do not seem to depress the inhibitory reflexes, and in most instances the action of the inhibitory reflex seems to be increased, although this may be only a relative result. Ether tensions that will depress the respiratory center so that it will not respond to the increase of carbon dioxide in the blood, usually will not abolish the inhibitory reflex. Under such conditions stimulation of the nerves inhibiting respiration will quite frequently produce death. This may be the process by means of which sudden death is produced during operation. However, death due to inhibition of respiration should never occur under light surgical anesthesia.

[2] In an excellent article on shock, Webster (5) shows a kymograph tracing in which pulling on the mesentery in a shocked animal produced inhibition of respiration and death.

[3] Hewitt (6) reports a case in which respiratory failure occurred during abdominal incision, but artificial respiration was effective. The corneal reflex had disappeared. Results in this case seem to parallel our experiment.

Fig. 13. Photograph of kymograph recorded showing slight recovery and then failure after removal of the ligatures. Animal etherized at 9:25; method—anesthetometer. The apparatus was arranged to record carotid blood pressure. The major artery to each limb was exposed. Record 1 taken at 9:45, blood pressure 128. Ligatures were applied to each limb including all structures except the major artery between 9:51 to 10:00. Record 2 taken at 10:00, blood pressure 108. Record 3 taken at 11:00, blood pressure 76. Animal beginning to exhibit the signs of shock. Record 4 taken at 11:30, blood pressure 45. The ligatures were removed at 11:35 to 11:40. Record 5 taken at 12:10, blood pressure 60. Record 6 taken at 1:10, blood pressure 50.

Fig. 14. Photograph of kymograph record showing partial recovery after removal of the ligatures. Animal etherized at 2:25; method—anesthetometer. The apparatus was arranged to record carotid blood pressure. The major artery to each limb was exposed. Record 1 taken at 3:04, blood pressure 118. Ligatures were immediately applied to each limb including all structures except the major artery. Record 2 taken at 3:15, blood pressure 110. Record 3 taken at 3:45, blood pressure 60. Animal beginning to exhibit the signs of shock. Record 4 taken at 4:45, blood pressure 56. Ligatures were removed immediately after taking this record. Record 5 taken at 12:15, blood pressure 88.

IV. THE RELATION OF THE CAPILLARY AND VENOUS BEDS TO THE SIGNS OF SHOCK

This investigation was made for the purpose of determining the smallest capillary and venous area which could be made to contain enough fluid to produce the signs of shock. There is no doubt that the cause of the condition which the surgeon calls shock is, in a large number of cases, a loss of circulating fluid. The method by means of which this fluid is lost to the circulation is not known nor is its place of sequestration fully established. It has been shown (7) that the capacity of the vascular system in the splanchnic area is such that it would hold several times the normal amount of blood. There is also no doubt but that in shock due to exposure of the abdominal viscera the initial loss of fluid takes p'ace in this area. What relation the remaining capillary and venous area of the body bears to the loss of circulating fluid is not known. Cannon (8) believes, and has presented data to substantiate the belief, that the lost fluid is due to capillary stagnation.

Janeway and Jackson (9) have shown that a circulatory failure which presents the typical signs of shock can be produced in dogs by a partial occlusion of the inferior vena cava at its point of entrance into the thorax. This has been corroborated by other observers.

The method of investigation in this series of experiments consisted in including in one single strong ligature all the structures to each limb except the major artery. In this way the major artery was allowed to pump blood into the limb from which all venous and lymph return was obstructed. The animal was maintained under a constant ether anesthesia and carotid blood pressure was recorded (mercury manometer). The ligatures were always applied so as to include as much of the limb as possible. Under such experimental conditions three results might occur: *a*, Stagnation of circulatory fluid in the occluded venous and capillary area to their full capacity. The general effects of this loss of fluid would depend on the size of the area involved and the ability of the remainder of the tissues to compensate for the loss. *b*, Injury, owing to lack of proper circulation, to all the tissues of this region. *c*, After removal of the ligatures, the passage into the general circulation of toxic products which might have been formed during the period of occlusion.

The results of a large series of experiments are in general agreement. The first result of ligating the limbs in the manner described is usually a slight and transient rise in blood pressure, although occasionally the

pressure may decrease from the beginning. In any event, the blood pressure soon decreases and at the end of two hours has only about one-half its initial value. Usually this decrease takes place very gradually but sometimes it drops suddenly after having been maintained at approximately its normal level for a long time. Other signs of shock also develop; for example, a short time after the application of the ligatures to the limbs it is usually possible to decrease the ether tension to a point greatly below that necessary to maintain anesthesia in a normal animal. Thus, at the end of two hours after ligation, an animal usually exhibits the major signs of shock.

If the ligatures are removed after blood pressure has decreased about one-half of its initial value, one of two results follows. Blood pressure either increases or decreases. In the majority of our experiments blood pressure increased after the removal of the ligatures, but complete recovery did not occur. This rise in pressure was seldom long maintained but soon decreased again and within an hour or so was as low as when the ligatures were removed. In some of the experiments the blood pressure fell immediately after the removal of the ligatures. In most of the animals death soon occurred although in a few the blood pressure was maintained at that low level for a long period of time. The results of the experiments may be best explained as follows: The initial fall in blood pressure is probably due to the occlusion and stagnation of circulatory fluid in the isolated capillary and venous areas. The variation in the length of time in which the blood pressure decreases apparently depends on the ability of the rest of the body to compensate for the fluid loss. The variable results following the removal of the ligature are due to damage to the tissues in the involved area and the passage of toxic products from the injured tissues into the general circulation.

The primary factors involved in these experiments, *a*, stagnation of circulatory fluid, *b*, damage to large areas of tissue in such a manner that their mechanism for controlling fluid exchange and, when the area is large enough, volume is impaired, and *c*, toxic products of cell metabolism and cell disintegration may all be of importance in the production of some of the conditions which the surgeon calls shock.

In such experiments it is difficult to make estimations of the relative capacity of the capillary and venous beds involved to the total capacity of all these beds in the body. However, it was possible to obtain approximate data on the relation of the total weight of tissue below the ligature to the total body weight. This was done in animals not sub-

jected to ligation in order to eliminate the possibility of changes in the tissues themselves as the means of complicating the results. The animals had been used in other experiments and had been fasted for twelve hours before death. The limbs were amputated at the level at which the ligature was applied and a comparison of the total weight of the amputated limbs to total body weight was made. As there are several sources of error, the comparisons are only approximate. The data show that on an average 15 per cent of the total weight of the tissues of the body was involved in the ligature.

Ligation of only three limbs produced variable results. Blood pressure was rarely lowered to a shock pressure. Section of the nerves to each limb did not seem to produce any change in the effect of the ligation.

In summarizing it may be restated that ligation of all the structures to the limbs of a dog except the major artery, will usually produce all the signs of shock. The relative amount of tissues involved by these ligatures was on an average approximately 15 per cent of the total body weight. The experiments show that a circulatory impairment following venous obstruction of the return of blood from the four limbs of an etherized animal is sufficient to produce the signs of shock.

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THE PHOTO-CHEMICAL EFFECT OF CERTAIN FLUORESCENT SUBSTANCES ON RENNIN

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INTRODUCTION

Von Tappeiner, Jodlbauer and their coworkers (1) have carried out a great number of experiments on the destructive effect of visible radiation on various cells, enzymes and toxins when fluorescent substances are present. Not all fluorescent substances are found to be consistently harmful and there is no correlation between the degree of fluorescence and the amount of photodynamic action for the different materials used. However, the fact that all the substances which were found harmful are fluorescent in some degree seems to establish a definite connection between the two effects. Two facts that stand out clearly are *a*, that only the wave-lengths absorbed by the fluorescent substance are harmful; *b*, that for visible rays the presence of oxygen is necessary to produce photodynamic action. The first fact would be true if the effect were due to any photochemical change and does not necessarily point to any connection with fluorescence. The second fact seems to indicate that the effect is photochemical and that fluorescence is not the essential factor, since oxygen is not necessary for fluorescence.

A number of experimenters have objected to the conclusion that the property of fluorescence is in itself harmful. Straub (2) found that the oxidation of many well-defined chemical substances, by the oxygen of the air, is greatly increased by eosin in the presence of light. He concluded that eosin is toxic on account of poisonous substances formed by autoxidation. Heffter (3) examined the acids formed by eosin in the process of autoxidation and found carbonic, oxalic, phthalic and hydrobromic acids. It would seem that the formation of these acids might easily explain the harmful effects on paramoecia and bacteria, especially in view of the Ledoux-Lebard experiment and von Tappeiner's addition to it. Ledoux-Lebard (4) found that a previously illuminated

solution of eosin was fatal to paramoecia in the dark, whereas a freshly prepared solution of the same strength was harmless. Von Tappeiner showed that this effect is definitely due to the formation of acids, as neutralizing the illuminated solution renders it harmless again. He is, however, convinced that the slow formation of free acids does not account for the swift photodynamic effect observed, especially as paramoecia will die in neutral and alkaline solutions in the presence of fluorescent substances and light. Also, a photodynamic death is followed by a swift disintegration of the paramoecia, whereas after a death from free acids they keep their shape for many hours.

Von Tappeiner's own theory attempts to explain the fact that while oxygen is necessary for a toxic effect with visible light, ultra-violet rays are harmful in its absence. He believes that the fundamental phenomenon, in both cases, is a decomposition by which groups are released which can be oxidized by light in the presence of oxygen. With short wave-lengths this decomposition is far-reaching under all conditions whereas, with visible light, it only reaches a perceptible degree when oxygen is present and the oxidizable decomposition products are continually removed by oxidation. The fluorescent substances, he believes, hasten the oxidation and therefore the whole process.

This explanation still leaves the rôle of the fluorescent substances in a very vague state and the following experiments were carried out in hopes of finding some clue as to what this rôle might be. To avoid complications from the toxic effects of acids, rennin was chosen as the subject for experimentation.

Rennin is favored by a slightly acid medium and is not killed by heat under a temperature of 55°C.

PREVIOUS WORK ON RENNIN

Without the presence of a sensitizing substance rennin is very sensitive to ultra-violet light and is affected very little if at all by visible rays. Emmerling (5) found that an exposure of five days to indirect sunlight cuts down the activity of rennin to one-half, exposure to direct sun to one-third. Hertel (6) found that the waves of λ (wave length) $280\mu\mu$ are very destructive to rennin and Schmidt-Neilson (7) found that 96 per cent of the total effect of a quartz mercury arc on rennin is due to waves of $\lambda\ (220 \rightarrow 250)\mu\mu$.

Riegner (8) and Quiring (9), two pupils of V. Tappeiner, investigated the effect of light on rennin in the presence of fluorescent substances. They found a strong inhibitive action in the presence of substances of

the fluorescein group and a measurable, though small, effect with the anthracene group, magdala red and methylene blue. Negative results were obtained with substances of the acridine, phenazin and napthalene groups, with quinoline dyes and aesculin.

Huber (10) in a paper on the sensitizing effect of fluorescent substances, gives some interesting experiments on the effect of eosin and erythrosin on rennin ferment. He gives the following results:

1. While a given rennin solution, containing eosin or erythrosin, is unaffected as long as it remains in the dark and will clot milk in ten minutes, on exposure to light its activity is decreased so that the clotting does not occur for an hour or more.

2. The same result, though to a very much smaller degree, was observed when the eosin or erythrosin was added to milk and exposed and the rennin added subsequently.

3. When the eosin or erythrosin was exposed alone, and then added to the milk and rennin, no weakening of activity was observed.

4. The presence of air favored the toxic effect of eosin and light but there was still a large effect when air was excluded.

5. Under screens of eosin and erythrosin solutions the effect of light is diminished but still large.

6. The toxic effect of light on eosin and rennin is permanent, since a solution exposed and kept overnight has the same action as one to which milk is added immediately after exposure.

METHOD

In the following experiments a solution was made from one Hansen's junket tablet to 30 cc. of distilled water. To 5 cc. of this solution definite amounts of solutions of eosin and other substances were added. The solution was exposed to direct sunlight in an open beaker and then added to 15 cc. of milk at a temperature of 30°C. The mixture was placed in a thermostat at 37°C. and the time of clotting observed. The controls of 5 cc. exposed junket solution and 15 cc. of milk clotted in 5 → 8 minutes.

In experiments with color screens of absorbing solutions, the rennin solution was placed in a small flat dish and a slightly larger dish, covered and filled with the absorbing solution, was placed on top. The sides of the bottom dish were blackened so that the light came only through the absorbing screen. With colored glass screens the upper dish was replaced by the glass.

RESULTS

The effect of heat was first tried and the results are given in table 1.

There is therefore no destructive effect from heat under 55°C. and as the experiments were conducted in cool weather the temperature,

TABLE 1

Effect of heat

	MINUTES TO CLOT
5 cc. rennin solution heated to 50°C.	8
5 cc. rennin solution + 0.0005 gram eosin heated to 50°C.	5
5 cc. rennin solution heated to 55°C.	8
5 cc. rennin solution + 0.0005 gram eosin heated to 55°C.	5
5 cc. rennin solution heated to 60°C.	No clot in 35 minutes
5 cc. rennin solution + 0.0005 gram eosin heated to 60°C.	

even in direct sunlight, was always under 30°C. and there were no complications from heat.

The effect of acid, alkali and alcohol is shown in table 2.

TABLE 2

Effect of acid, alkali and alcohol

	MINUTES TO CLOT
5 cc. rennin solution	5→7
5 cc. rennin solution + 0.5 cc. 0.1 per cent NaOH	12
5 cc. rennin solution + 1.0 cc. 0.1 per cent NaOH	20
5 cc. rennin solution + 0.5 cc. 0.1 per cent H_2SO_4	4
5 cc. rennin solution + 1.0 cc. 0.1 per cent H_2SO_4	3
5 cc. rennin solution + 0.2 cc. 1 per cent H_2SO_4	2
5 cc. rennin solution + 0.4 cc. 1 per cent H_2SO_4	1
5 cc. rennin solution + 0.5 cc. 95 per cent alcohol	5
5 cc. rennin solution + 1.0 cc. 95 per cent alcohol	5

Since the activity of rennin is favored by an acid and hindered by an alkaline medium, and unaffected by alcohol, it was possible to try the effect of several substances that are soluble in alcohol and not in water.

The fact that eosin is harmless except on exposure to light is shown in table 3.

TABLE 3

Effect of eosin in dark

	MINUTES TO CLOT
5 cc. rennin solution in dark 5 hours	7
5 cc. rennin solution + 0.0005 gram eosin in dark 5 hours	7
5 cc. rennin solution + 0.001 gram eosin in dark 5 hours	7
5 cc. rennin solution + 0.0005 gram eosin in dark 48 hours	8
5 cc. rennin solution + 0.001 gram eosin in dark 48 hours	8

Huber (10) found that previously illuminated eosin is harmless and that the toxic effect resulting from exposure of eosin and rennin is permanent. These conclusions are confirmed by the results given in tables 4 and 5.

TABLE 4

Effect of previously illuminated eosin

	MINUTES TO CLOT
0.001 gram eosin (water solution) $3\frac{1}{2}$ hours sun (rennin added later).	6
0.001 gram eosin (water solution) $1\frac{3}{4}$ hours sun (rennin added later).	7

TABLE 5

Permanency of toxic effects

MILK ADDED AT ONCE	TIME TO CLOT	MILK ADDED AFTER STANDING OVER NIGHT	TIME TO CLOT
5 cc. rennin solution + 0.0005 gram eosin, 1 hour sun	$1\frac{3}{4}$ hours	Same	$1\frac{3}{4}$ hours
5 cc. rennin solution + 0.001 gram eosin, 1 hour sun	$3\frac{1}{2}$ hours	Same	$3\frac{3}{4}$ hours
5 cc. rennin solution + 0.001 gram fluorescein, $2\frac{1}{2}$ hours sun	50 minutes	Same	50 minutes
5 cc. rennin solution + 0.001 gram methylene blue, $2\frac{1}{2}$ hours sun	$1\frac{1}{2}$ hours	Same	2 hours

After these preliminary experiments special ones were undertaken with a view to determining what rôle fluorescence plays in the effect.

If fluorescence is the determining factor one would expect the following results:

1. All fluorescent substances should be harmful, when wave-lengths capable of stimulating fluorescence are used, and non-fluorescent substances should be harmless.

2. A strongly fluorescent substance should be more toxic than one in which the fluorescence is weak or barely perceptible.

3. The wave-lengths of light which stimulate maximum fluorescence should give the maximum toxicity.

To test the first point a number of substances were examined. The following gave negative results: Congo red, methyl green, fuchsine, neutral red, methyl orange, chlorophyll (alcoholic solution), quinine sulphate (faintly acid solution), gentian violet, resorcinol and haematoporphyrin. Of these, chlorophyll and quinine sulphate show strong fluorescence; congo red, neutral red and haematoporphyrin weak fluorescence. Toxicity in the presence of light was found to be very marked for eosin and erythrosin and present, though weak, for fluorescein and methylene blue all of which are fluorescent. It was also found, as will be given in detail later, that a non-fluorescent emulsion of silver bromide and rennin solution was toxic in the light. So all fluorescent substances are not harmful and all non-fluorescent substances are not harmless.

As to the second point, experiments have been carried out by V. Tappeiner (1) on the relation between the degree of fluorescence and the amount of toxicity for paramoecia. He found that, with any given substance, the toxicity changed in the same direction as the fluorescence. For example, they both decrease with the addition of salt to quinine sulphate. But, except for this, there was no connection. Fluorescein is very fluorescent and not very harmful while the opposite is true of anthraquinone.

And, in the case of substances of the fluorescein group, the toxicity actually increases with decrease in fluorescence as is shown in the following table from Von Tappeiner's work (11) (table 6).

I obtained a similar result with the fluorescein groups and rennin (table 7).

Fluorescein shows even less toxicity than methylene blue, a weakly fluorescent substance of another group (see table 8).

So far there would seem to be no evidence that fluorescence is the controlling factor in photodynamic action on rennin. The third point to be tested is, however, the decisive one and, in the case of eosin, the wave-lengths giving maximum fluorescence are well known. Nichols and Merritt (12) made a careful study of the fluorescent spectra of a number of substances with the spectrophotometer in order to test the validity of Stokes' law (i.e., the fluorescent light is always of greater wave-length than the exciting light). Among these substances they

tried eosin in dilute alcoholic solution. Their curves showing the distribution of intensities in the fluorescent spectrum, for different wave-lengths of exciting light, are reproduced in figure 1. The maximum

TABLE 6

SUBSTANCE	BRIGHTNESS OF FLUORESCENCE	TOXICITY TO PARAMOECIA IN DIFFUSE DAYLIGHT (THAT IN DARK = 1)	TOXICITY TO INVERTIN IN PER CENT
Fluorescein	Very strong	11	0
Tetrachlor fluorescein	Strong	35	10
Tetrabrom fluorescein (eosin)	Moderate	60	57
Tetraiodo fluorescein (erythrosin)	Weak	80	89
Dichlortetrabrom fluorescein	Only perceptible with lens in sun	150	89
Dichlortetraiodo fluorescein		100	96
Tetrachlortetraiodo fluorescein		170	89

TABLE 7

	EXPOSURE TIME	
	15 minutes	45 minutes
	TIME TO CLOT	
5 cc. rennin solution + fluorescein 0.001 gram	8 minutes	12 minutes
5 cc. rennin solution + erythrosin (tetraiodo fluorescein) 0.001 gram	12 minutes	45 minutes
5 cc. rennin solution + eosin (tetrabrom fluorescein 0.001 gram	15 minutes	No clot in 6 hours

TABLE 8

	EXPOSURE TIME	
	$1\frac{1}{2}$ hours	$2\frac{1}{2}$ hours
	TIME TO CLOT	
5 cc. rennin solution + fluorescein (0.001 gram)	20 minutes	50 minutes
5 cc. rennin solution + methylene blue (0.001 gram)	30 minutes	$1\frac{3}{4}$ hours

wave-length of the fluorescent light and the distribution of intensity in the fluorescent band, are independent of the wave-length of the exciting light, but the maximum intensity of fluorescent light for eosin is given

by λ 590 + $\mu\mu$ for the exciting light which is greater than 580$\mu\mu$, the wave-length of maximum intensity of fluorescent light (a contradiction of Stokes' law). With decreasing wave-lengths the intensity of the fluorescent light diminishes.

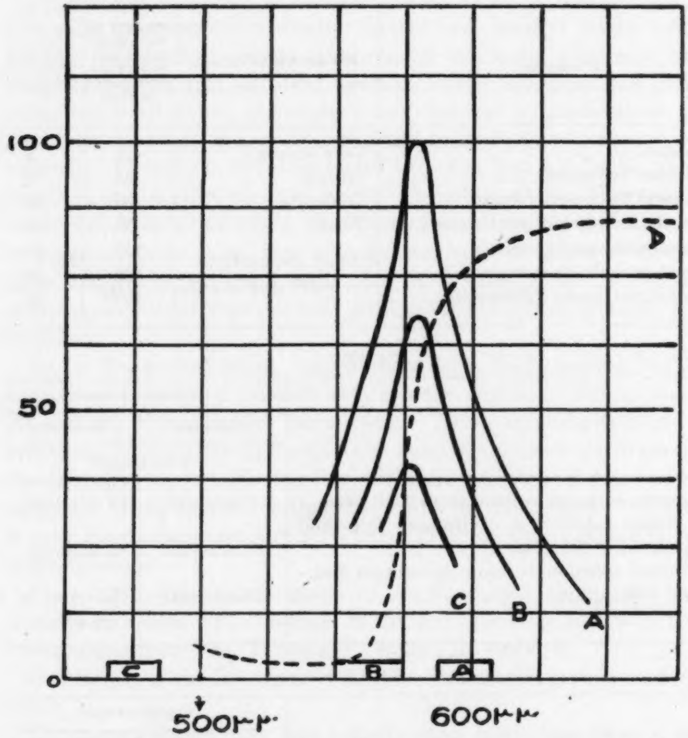

Fig. 1. The fluorescence spectrum of eosin when the exciting light lies in different regions of the spectrum. Ordinates give the intensity of fluorescence and abscissae the wave-lengths. Curve A was obtained when the exciting light was confined to the region marked A on the axis of wave-lengths. D = Transmission curve of eosin. Vertical scale arbitrary. Horizontal scale in $\mu\mu$ = 0.000001 mm.

Huber, in his experiments with rennin and eosin, found that an eosin screen did not cut down the effect of light to any great extent. This is interesting as, in general, for any photochemical or fluorescent effect, one would expect only the wave-lengths absorbed by the sub-

stance itself to be effective. However, since the results of Nichols and Merritt show that the maximum fluorescence of eosin is caused by wave-lengths just to the red of the absorption band, the results given by Huber may not be in disagreement with the theory that fluorescence is the controlling factor in photodynamic action on rennin. In order to find just which wave-lengths are most injurious, 5 cc. of rennin solution + 0.001 gm. of eosin or erythrosin, was exposed to sunlight behind screens of methyl red, potassium bichromate, chrome alum, cuprammonium and eosin (or erythrosin). These substances have the following transmission in the visible.

Methyl red	$\lambda\lambda$ 600 $\mu\mu \rightarrow$ 690 $\mu\mu$
Potassium bichromate	$\lambda\lambda$ 500 $\mu\mu \rightarrow$ 690 $\mu\mu$
Chrome alum	$\lambda\lambda$ 450 $\mu\mu \rightarrow$ 550 $\mu\mu$
Cuprammonium	$\lambda\lambda$ 420 $\mu\mu \rightarrow$ 520 $\mu\mu$

Eosin, in moderately dilute solutions, cuts off everything below λ 550$\mu\mu$ in the visible, but, with a very dilute solution, there is partial transmission from λ 460$\mu\mu$ to λ 420$\mu\mu$.

Erythrosin, moderately dilute, cuts out everything below λ560$\mu\mu$ but, when more dilute, gives weak transmission from λ420$\mu\mu$ to λ480$\mu\mu$.

The glass screens used were more dense than the solution screens and required longer exposure. They showed the following transmission:

Red glass	$\lambda\lambda$ 680 $\mu\mu \rightarrow$ 600 $\mu\mu$
Yellow glass	$\lambda\lambda$ 680 $\mu\mu \rightarrow$ 510 $\mu\mu$
Green glass	$\lambda\lambda$ 620 $\mu\mu \rightarrow$ 480 $\mu\mu$
Blue glass (cobalt)	$\lambda\lambda$ 510 $\mu\mu \rightarrow$ 420 $\mu\mu$
With a faint transmission band at	λ 600 $\mu\mu$

If the effect is one due to fluorescence the maximum effect for eosin would be expected behind the potassium bichromate screen. There is unfortunately no definite investigation giving the maximum stimulating wave-length for erythrosin. It has a yellowish-green fluorescence and the stimulating wave-lengths would probably lie in the green and blue. Experiments with the color screens give the results of tables 9, 10, 11 and 12.

As eosin has no absorption band in the ultra-violet, one would expect no greater inhibition in that region with eosin and rennin than with rennin alone. Experiments with a quartz mercury arc confirmed this.

No color screen experiments were tried with fluorescein and methylene blue as they showed such a small effect. With erythrosin and eosin the maximum inhibition was found in the blue and green, green

TABLE 9

Eosin (0.001 gram in 5 cc. rennin solution) solution screens

	EXPOSURE TIME							MEAN
	2 hours sun	2 hours sun	1½ hours sun	1½ hours sun	1½ hours sun	1½ hours sun	1½ hours sun	
	Clotting time (minutes)							
Red screen	13	5	10	12	12	12	13	11
Yellow screen	19	9	30	22	60	20	30	27
Green screen	25	13	45	45	240	110	60	77
Blue screen	90	35	50	60	300	85	210	119
Eosin screen	35	28	15	15	12	15	18	20

TABLE 10

Eosin (glass screens)

	EXPOSURE TIME				MEAN
	2½ hours sun	3 hours sun	2 hours sun	2 hours sun	
	Clotting time (minutes)				
Red	10	9	9	9	9
Yellow	10	15	10	11	11
Green	90	50	20	20	45
Blue	270	180	25	25	125

TABLE 11

Erythrosin (0.001 gram in 5 cc. rennin solution) solution screens

	EXPOSURE TIME								MEAN
	1½ hours sun	1½ hours sun	3 hours moderate sun	3½ hours moderate sun	4 hours sun	1½ hours bright, 2 hours dull	2½ hours sun	2 hours sun	
	Clotting time (minutes)								
Red	15	10	8	13	30	20	8	12	15
Yellow	15	10	10	15	55	30	35	30	25
Green	20	10	18	35	170	30	190	105	72
Blue	18	15	15	25	210	38	70	60	56
Erythrosin	18	10	10	13	40	18	30	35	22

light being most effective for erythrosin and blue for eosin. In the case of erythrosin this would be consistent with the theory that fluorescence is the primary cause of inhibition. The results with eosin, however, show very definitely that this is not true as the most marked inhibition is due to blue light, which gives but faint fluorescence, and the yellow light that stimulates maximum fluorescence is almost harmless. No attempt was made to determine the relative amounts of energy transmitted by the different screens.

TABLE 12

Erythrosin (glass screens). Exposure time 4 hours sun

	CLOTTING TIME (MINUTES)					MEAN
Red	5	5	8	10	8	7
Yellow	10	8	8	20	8	11
Green	25	10	30	15	16	19
Blue	15	12	20	25	12	17

DISCUSSION

There is unfortunately no generally accepted theory of fluorescence. The most comprehensive one so far proposed is that first suggested by Wiedemann (13) in 1889, and later modified and extended by Wiedemann and Schmidt (14). According to this theory some chemical or physical change is produced in a luminescent substance during excitation and the emission of light is an accompaniment of a more or less gradual restoration of the modified substance to its original condition. That is, under the action of light, a portion of the substance is changed from a stable condition A to an unstable condition B. Luminescence may be due to the vibrations set up during the change $A \rightarrow B$ or to the fact that the reaction $B \rightarrow A$ proceeds, during excitation, with the emission of light. According to this theory fluorescence is primarily a photochemical phenomenon. It would seem reasonable to suppose that, while in the unstable state B, some fluorescent substances might be harmful and some not, depending on what by-products were formed in the changes $A \rightleftarrows B$. In this case one might look for a harmful photochemical effect in both fluorescent and non-fluorescent substances, but with the chances in the favor of effective substances being fluorescent, since all fluorescent materials are subject to photochemical action.

A very familiar photochemical reaction is that which takes place with a photographic plate. A plate coated with silver bromide alone.

and exposed to light is acted on to form free bromine but, when a certain amount is formed, a stationary balanced condition is reached owing to the recombination of silver and bromine. Such a plate would be of very little use in photography, being comparatively insensitive. If, however, a substance such as gelatine is present, which combines with the bromine as it is formed, a much greater decomposition of the silver bromide takes place.

Probably the action of light on eosin is entirely similar. When exposed to light it undergoes the transformation $A \rightleftarrows B$. Unless some substance is present to take up the harmful dissociation products as they are formed, the reaction soon reaches equilibrium and little, if any, permanently toxic substance is formed. This would explain the fact that previously illuminated eosin is harmless (see table 4). If however eosin and rennin are exposed together the rennin, acting like the gelatine of the photographic plate, takes up the toxic substances as they are formed and the reaction proceeds much further.

TABLE 13

	CLOTTING TIME
5 cc. rennin solution	10 minutes
5 cc. rennin solution + 0.5 gram AgBr 3 hours dark	10 minutes
5 cc. rennin solution + 0.5 gram AgBr 20 hours dark	10 minutes
5 cc. rennin solution + 0.5 gram AgBr 3 hours moderate sun	1½ hours
5 cc. rennin solution + 0.5 gram AgBr 3 hours bright sun	No clot in 5 hours

Now eosin and erythrosin are both very toxic whereas fluorescein, from which they are derived, is comparatively harmless. Since eosin is tetrabrom fluorescein and erythrosin is tetraiodo fluorescein, the presence of the halogens must account for the difference. Probably the action of light results in freeing bromine and iodine, or some simple compound of these substances and these, being taken up by the rennin as they are formed, destroy its activity.

The experiment was tried of adding free bromine and iodine to the rennin solution and in both cases its activity was greatly inhibited, especially in the case of bromine where the smallest trace produced a strong effect. If the inhibition is due to the action of free halogens on rennin one would expect an emulsion of silver bromide to be harmful to rennin in the light and not in the dark. Experiments with silver bromide gave the following results which definitely confirm the view given above (table 13).

A direct proof of the formation of free iodine from erythrosin under the action of sunlight was tried by adding starch to a solution of erythrosin both with and without the rennin. The results however were negative as shown in table 14.

This probably means that in the case of erythrosin, the harmful dissociation product formed, under the action of sunlight, is not the free halogen but a toxic halogen compound.

TABLE 14

RENNIN SOLUTION	ERYTHROSIN	STARCH SOLUTION 2 PER CENT	RESULT (16 HOURS SUN)
cc.	*grams.*	*cc.*	
5	0.001	5	No evident darkening
5	0.010	5	No evident darkening
5	0.100	5	No evident darkening
5 (distilled water)	0.010	5	No evident darkening
5 (distilled water)	0.100	5	No evident darkening

CONCLUSION

These results seem to show that the inhibition of rennin by light is due to the formation of toxic substances as the result of photochemical action. This photochemical action may or may not be accompanied by fluorescence and fluorescence, always an evidence of photochemical action, may or may not be accompanied by the formation of toxic substances.

In the cases studied here the toxic effect was probably due to the formation of free halogens or toxic halogen compounds though doubtless other harmful substances are found in different photochemical reactions. Although the formation of free halogens or toxic halogen compounds accompanies the fluorescence of eosin and erythrosin, it is not directly associated with it, as shown by the color screen experiments with eosin. The change $A \rightleftarrows B$, which gives rise to fluorescence, is due primarily to the greenish-yellow and yellow light for which eosin shows the greatest absorption. The change which results in the formation of free halogens is apparently a subsequent $B \rightleftarrows C$ or accompanying $A \rightleftarrows C$ effect due primarily to greenish-blue and blue light for which eosin shows partial absorption. This accounts for the fact that, though strong solutions of eosin form an effective screen and keep the exposed solution from being inhibited, a weak solution, which lets through some radiation in the region $\lambda\ 460\mu\mu$ to $\lambda\ 420\mu\mu$, will allow considerable inhi-

bition (see color screen results and Huber). In the absence of some substance, such as rennin, to take up C there is probably little reaction $B \rightleftarrows C$ or $A \rightleftarrows C$ present, and the chief photochemical change taking place is $A \rightleftarrows B$, which is accompanied by fluorescence.

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CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. NO. 312.

THE NATURE OF SENSORY STIMULATION BY SALTS

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The purpose of this investigation is to determine as far as possible the nature of the effect of salt solutions on the chemical sense of the dung-worm, Allolobophora foetida. First of all it is necessary to show whether or not stimulation by some salts is dependent on the anions, since investigations so far have been in favor of cations only. It has been found that in the case of four salts the cations were responsible for stimulation; the order of their efficiency was as follows: $KCl < LiCl < NH_4Cl < NaCl$ (Parker and Metcalf, '06). In man, however, the sense of taste as regards salt solutions is found to be dependent upon cations in some instances and upon anions in others; for example, with NaCl, the chlorine ion is responsible for the salty taste, while with KCl, the action of the cation is masked by the potassium ion, which gives a bitterish taste. Therefore, on further investigation it may be possible to find that in dung-worms there may be anions that are efficient enough to mask the effect of the cations.

To carry out these tests an apparatus consisting of a small circular glass-table standing in a dish of the solution to be used in the test was employed (fig. 1). The top of the table, *T*, was 9 cm. in diameter, and was coated with paraffin. It was attached firmly to a stand, *S*, made of a small Petri dish 2.6 cm. in height, 3.5 cm. in diameter and filled with paraffin. This stand was fixed to the center of a large Petri dish, *D*, 15 cm. in diameter and 3.5 cm. in height. The paraffin used had a melting point of 62°C. and was tested for its acidity by the method devised by Haas ('16) for the determination of the output of carbon dioxide by plants. In preparing to test the worms, about 450 cc. of the liquid to be used was poured into the large Petri dish till its level, *L*, was within 3 mm. of the top of the table. A wet filter paper with a

smooth surface was then placed on the table and the whole apparatus was put in a tin dish containing enough water to rise on the outside of the large Petri dish about 2.5 cm. The temperature of the room and of the whole apparatus was kept approximately constant and within one degree of 20°C. The worm was then gently placed on the table by means of a chopstick and allowed to crawl toward the edge and finally to enter the solution. The period intervening between the moment the prostomium of the worm entered the solution and that at which it was withdrawn was recorded by a stop-watch. This interval is called the reaction-time of the worm to the solution. After the worm had been once placed on the table, it was not touched by the experimenter till an observation had been made. After that it was removed from the table, rinsed in tapwater and kept between folds of wet filter paper in a tumbler until the next observation was to be made.

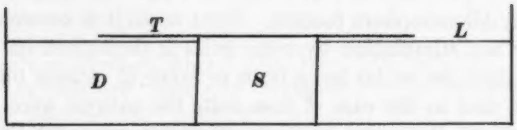

Fig. 1. Vertical section of the apparatus used in determining the worm's responses to various fluids. *D*, large Petri dish; *L*, level of fluids; *S*, stand; *T*, table covered with paraffin.

The various worms were found to differ in their reaction-times one from another to such an extent that it was impossible to draw directly any safe conclusions as to the relative efficiency of the different salt solutions without some method of standardization. A 0.2 molecular solution of KCl was therefore used as a standard solution, the reaction-time from it being regarded as 10 seconds. By this means variations due to the individual differences of the worms were largely eliminated.

To prevent the sequence in which the worms were tested and the order in which the solutions were used from distorting the results, the tests were made in the order of the numbers in the following scheme arranged for three worms and three solutions with an interval of fifteen minutes between observations to avoid as much as possible the after effect of the previous treatment with salt solution.

WORM DESIGNATED BY LETTER	STANDARD SOLUTION	TEST SOLUTION NO. 1	TEST SOLUTION NO. 2
A	1	2	3
	17	18	16
	24	22	23
B	6	4	5
	10	11	12
	27	26	25
C	8	9	7
	15	13	14
	19	20	21
	Averages	Averages	Averages

In obtaining a set of records by such a scheme as this, twelve worms of equal size were chosen, three to be used in each of four sets, whose averages were calculated separately. If the averages for the four sets agreed within the experimental error, they were averaged together and from such final averages curves were drawn.

The general method here outlined has the following advantages over methods used by previous experimenters. First, since the worm always enters the solution by extending the anterior end of its body, its prostomium, which is its most sensitive part, is always fully exposed to the solution. The records obtained are, therefore, records of the effect of the salts on the extended prostomium. Secondly, this method avoids handling the worm immediately before a reaction-time is taken. Thirdly, the response observed represents very closely a true reaction-time, since there are no mechanical obstructions for the worm to overcome as with the fence-method devised by Shohl ('14).

To test the efficiency of the anions, six potassium salts were chosen; namely, the nitrate, chloride, acetate, sulphate, tartrate and citrate. The acetate, tartrate and citrate were tested for their alkalinity by the following method. The standard solutions with a known amount of indicator, phenolsulphonephthalein, were made up by different mixtures of buffer solutions to represent different pH values. The same amount of the indicator was added to the test solution and the color was matched with the standard solutions. In this way it was possible to determine how much acid to add in order to bring the pH value to 7.0. The amount of acetic, tartaric or citric acid added for the neu-

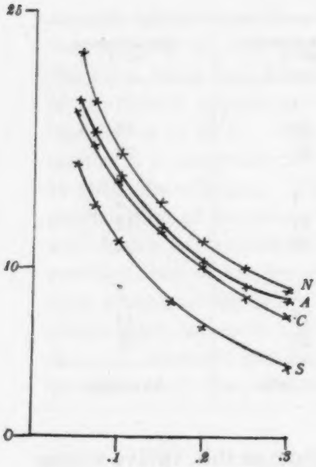

Fig. 2

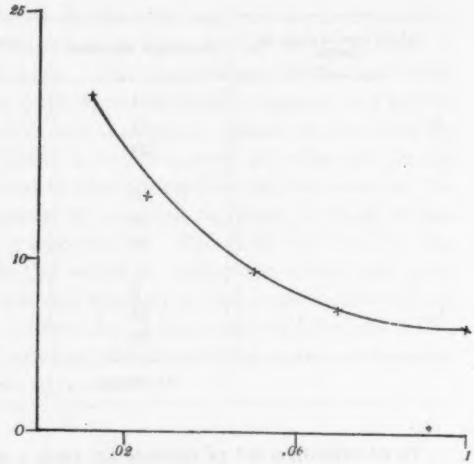

Fig. 3

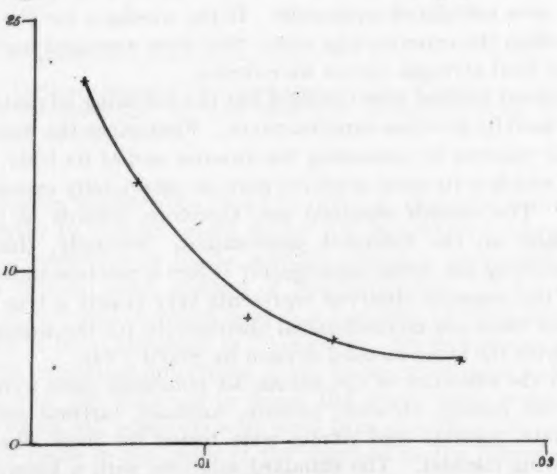

Fig. 4

Fig. 2. Relative efficiency of four potassium salts as stimuli for worms. The relative reaction-time in seconds of the worms to the solutions is applied as the ordinates and the molar concentrations as the abscissae. *N*, nitrate; *C*, chloride; *A*, acetate; *S*, sulphate.

Fig. 3. Effect of potassium tartrate. Relative reaction-time in seconds is applied as the ordinates and the molar concentrations as abscissae.

Fig. 4. Effect of potassium citrate. The relative reaction-time in seconds is plotted as the ordinates and the molar concentrations as abscissae.

tralization was not enough to change the dissociation percentage of the salts sufficiently to interfere with the experiments. Figures 2, 3 and 4 show the efficiency of the six potassium salts tested. The relative reaction-times in seconds are applied as ordinates and the molar concentrations as abscissae. At the concentration that brings about a relative reaction-time of about twenty seconds, the worms are fairly indifferent to the solutions, so that the relative reaction-times become too inconstant for an accurate computation. On the other hand at the concentration that gives the relative reaction-time of nine seconds, the reactions become so rapid that it is difficult to record them without considerable error. For this reason, in drawing comparisons of the efficiency of six salts it was necessary to choose concentrations which brought about relative reaction-times ranging from nine to sixteen seconds. On interpolating eight points between nine and sixteen seconds, and calculating the relative number of potassium ions from the dissociation percentages of a given salt, it is made evident that in case of nitrate, acetate, chloride and sulphate the number of potassium ions is approximately equal; therefore in these salts the potassium must be responsible for the stimulation. However, with the tartrate and the citrate, the amount of potassium ions is in all probability too small to attribute the effect to the cations.

The nature of the action of the solutions of potassium salts as dependent on the amount of the reacting substance present is susceptible of further proof as seen in the successive exposures of the worms to the potassium salt solutions, sucrose solutions and water (both tap and distilled). The only modification of the method described in the earlier part of the paper consisted in using for these tests a broader and deeper dish to hold the solution and a higher stand to carry the table. These changes prevented the worms from coming in contact with any part of the dish even when distilled water or a solution of sucrose was used, to which the worms react more or less indifferently. In making the tests, the worms were allowed to dip in and out of the solution in succession with an interval of less than ten seconds; the reaction-times, as in previous tests, were taken with a stop-watch. On comparing the reactions thus obtained with those to salt solutions, sucrose solutions and water, two types may be distinguished. One type is characteristic of those concentrations of the potassium salts that bring about an initial acceleration in the reaction-time, as indicated for 0.1 molecular potassium chloride solution in table 1. Here it will be seen that the reaction-times are at first long and become successively shorter and shorter.

TABLE 1

Reaction-time in seconds to successive applications of solutions of potassium chloride, of solutions of sucrose, and of distilled water. All fluids used were at 20°C. except the distilled water in the last series in the table; this was used at 33°C. The sign ∞ indicates that the worm entered the fluid instead of withdrawing from it.

	REACTION NUMBER																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
0.1 M. KCl	45.0	6.2	4.8	1.8	3.0	6.8	1.8	2.4	4.0	0.6	0.4	1.0	0.8	1.8	0.4	0.4	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
0.01 M. KCl	∞	40.0	2.8	2.2	6.4	3.0	4.2	∞	3.8	3.2	2.8	1.8	3.8	6.8	8.0	5.0	8.0	7.0	13.0	∞	4.0	2.8	2.8	4.0
1 M. sucrose	11.0	8.8	3.8	3.0	4.0	6.2	2.0	4.0	2.2	1.8	3.2	2.6	2.8	3.0	3.0	1.8	3.4	1.8	1.0	1.8	2.4	2.2	1.0	2.0
0.5 M. sucrose	20.0	4.0	6.4	20.4	3.4	5.4	4.4	3.0	3.0	7.0	5.6	5.4	3.2	13.0	6.0	1.8	3.8	3.0	5.8	3.0	2.8	4.8	2.8	1.8
H_2O, 20°C	7.0	4.6	4.0	2.0	1.8	1.2	3.0	1.8	1.4	2.6	8.8	13.0	2.0	1.8	1.8	2.0	2.0	1.8	4.0	4.2	1.8	1.0	2.2	3.0
H_2O, 33°C	6.8	4.4	4.2	2.8	3.2	3.2	3.2	5.4	3.0	2.0	2.8	2.0	2.8	1.8	1.4	1.8	2.0	1.8	2.0	1.8	3.0	2.8	2.6	2.8

	REACTION NUMBER																						
	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
0.1 M. KCl	0.2	0.2	0.2	0.2	0.2	0.2	0.4	0.4	0.4	0.4	0.4	0.6	0.8	3.8	8.0	8.2	1.2	0.8	8.4	4.0	1.2	2.8	7.2
0.01 M. KCl	20.0	6.4	17.8	3.0	2.8	2.2	3.0	6.8	3.0	3.0	6.8	4.0	3.8	3.2	1.8	30.0	3.8	2.8	3.0	∞	3.8	2.8	5.0
1 M. sucrose	1.0	2.0	2.0	2.0	5.4	2.4	1.2	1.2	1.2	2.0	2.4	4.0	4.4	1.4	2.0	1.8	6.0	5.0	2.8	2.4	3.0	1.8	6.0
0.5 M. sucrose	14.0	3.4	2.2	5.0	3.0	1.8	2.4	2.0	52.0	4.6	2.2	5.4	2.0	3.6	4.0	3.2	2.2	1.8	1.6	3.2	2.4	2.4	6.4
H_2O, 20°C	2.8	9.0	9.0	37.2	4.6	1.8	2.0	1.8	2.0	1.8	1.8	2.0	1.8	1.8	2.0	1.8	1.2	2.0	2.4	7.2	1.4	2.0	21.0
H_2O, 33°C	2.4	4.2	5.0	9.0	1.0	1.4	5.8	1.8	1.8	2.0	1.8	2.2	1.2	1.8	1.2	1.2	1.4	2.2	2.0	2.0	2.0	2.0	1.8

These two types are easily contrasted if the rates $\left(\frac{100}{\text{Reaction-time}}\right)$ of the successive reactions are plotted in sequence (figs. 5 and 6). The

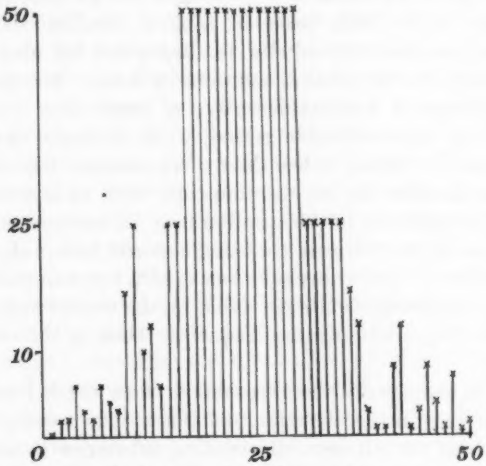

Fig. 5. Successive reactions of worms showing initial acceleration. This type of reaction is characteristic of the stronger concentrations of potassium salts. This graph is drawn from the first series in table 1 (0.1 molecular potassium chloride). The rates $\left(\frac{100}{\text{Reaction-time}}\right)$ are plotted as ordinates, and the sequence of reactions as abscissae.

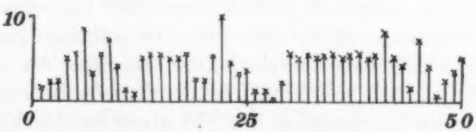

Fig. 6. Successive reactions of worms showing no initial acceleration. This type of reaction is characteristic of the weaker concentrations of potassium salts, of sucrose solutions and of water. This graph is drawn from the fifth series in table 1 (water 20°C.). The rates $\left(\frac{100}{\text{Reaction-time}}\right)$ are plotted as ordinates and the sequence of reactions as abscissae.

first type is illustrated in figure 5, which is a plotting of the rates of the successive reaction-times in the first series of table 1, viz., 0.1 molecular potassium chloride. The reaction is at first slow but after a few such

slow reactions, acceleration takes place and the reactions become very rapid. An equilibrium is then established for a number of successive reactions, after which the slow rates gradually reappear. The second type is represented in figure 6, a plotting of the rates of the successive reaction-times in the fifth series in table 1, distilled water at 20°C. This type is illustrated not only by distilled water but also by the solutions of sucrose and the weaker solutions of salts. To these fluids the worms react more or less indifferently; at times they will remain in the solution for a considerable period, while in many cases they will withdraw from the liquid in less than three seconds; but there is never a definite acceleration in the reaction-time such as is seen in the first type. This irregularity in the reaction may be interpreted as meaning that these non-aquatic worms are being exposed to liquids of minimum stimulatory effect. In weaker solutions of salt, for example 0.01 molecular solution of potassium chloride (table 1), the worms make a complete entrance into the solution more frequently than is the case with distilled water.

Although in some series the experiments were carried as far as three hundred reactions, no acceleration took place, thus proving that at such a concentration the amount of reacting substance is insufficient to bring about the type of reaction characteristic of the stronger concentration of the salt solutions before the appearance of the muscular fatigue which would naturally prevent any further continuation of the experiment.

Furthermore the above experiments with sucrose and water show that osmotic pressure is not a significant factor in the sensory stimulation of the worms, since all yielded essentially the same result. Of much more importance is the fact that the solutions just mentioned show a marked difference in relation to the effect on the worms as compared with the salt solutions. What, then, is the cause of such a difference? Is the acceleration brought about by the direct combination of the receptive protoplasm with the salts or by the substance formed as a result of muscular activity? To test this, the worms were tried out under different temperatures. At 40°C. the worms reacted to distilled water by instant withdrawal as if it were a very strong solution of salt, for example, 0.5 molecular potassium chloride. A temperature was therefore chosen at which the worms did not react instantaneously but at which their muscular activities were considerably greater than at the temperatures in which the former experiments were carried out. On successive exposure of the worms to distilled water

at 33°C. (table 1), while the room temperature was 20°C., no initial acceleration in the reaction-time took place, but a type of reaction similar to that brought about by the distilled water and sucrose solutions at 20°C. occurred. This indicates that the acceleration is not due to the products of muscular activity but must result from the specific action of the salt on the protoplasm of the sensory cells.

This conclusion is further supported by the following experiments. When a worm that had been stimulated by a salt solution to the point at which its reaction-time was shortest was transferred to a dish containing distilled water of the same temperature as that of the salt solution, its reactions were no longer nearly instantaneous, but were like those brought about by distilled water under ordinary conditions (table 2, worm A). But when the same worm was again transferred to the salt solution the very short reactions characteristic of the action of the salt was at once resumed. In this way the worm was transferred back and forth several times with the same result. On the other hand when a worm (table 2, worm B) was first exposed to distilled water for about twenty-five reactions and then transferred to the salt solution, the instantaneous reaction did not take place at once but appeared only after the worm had been dipping in and out of the salt solution for about twelve reactions. Thus it is clearly seen that the acceleration is brought about only by the action of the salt at proper concentration and not by distilled water. Furthermore, the reaction-time once accelerated by the action of the salt cannot persist except in the presence of the stimulating medium; when the salt solution is replaced by distilled water, the acceleration of the reaction at once disappears and is resumed only in presence of the salt solution.

When the same method of procedure was applied in the case of the weaker solutions of salt, for example, 0.01 molecular potassium chloride, into which the worms, as has already been mentioned, make complete entrance much more frequently than into distilled water, the difference in the reaction between such concentrations of salt and distilled water was clearly marked (table 2, worms C and D).

Thus it is made evident by the above experiments that sensory stimulation by potassium salts is due to the specific action of the salt, dependent essentially on the amount of the reacting substance present, and furthermore that such action may be distinguished from that of distilled water and sucrose solution.

The writer acknowledges her indebtedness to Dr. G. H. Parker, under whose supervision this work was carried out.

TABLE 2

Reaction-time in seconds of worms A, B, C and D to successive applications of pairs of stimulating fluids. Each pair consisted of water and some concentration of potassium chloride. Each worm was tested for a time first with one fluid, then with the other, and so on. The sign ∞ indicates that the worm entered the fluid instead of withdrawing from it

WORM		REACTION NUMBER 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
A	0.1 M. KCl..	∞	1.8	∞	1.0	∞	0.8	0.8	0.8													0.4	0.4	0.2	0.2	0.2		
	H_2O........									1.0	1.0	4.8	2.8	1.8	3.2	3.2	1.8	1.0	1.0	1.8	1.4						0.1	14.2
B	H_2O........	3.4	3.0	4.0	2.0	35.0	35.0	6.0	2.4	4.0	2.8	3.0	2.0	4.0	2.8	2.8	1.0	2.0	6.4	2.8	3.8	2.8	1.8					
	0.1 M. KCl..																							6.4	4.0	2.0	6.8	5.0
C	0.01 M. KCl.	∞	∞	1.8	2.0	60.0	4.0	4.2	8.0	3.4	10.0	5.0	16.4	5.8	∞	∞	6.8	4.8	3.2	6.2	2.8	6.8	∞	2.8	1.8	1.8	1.8	24.0
	H_2O........																											
D	H_2O........	12.0	5.0	15.0	3.0	4.0	6.4	2.2	12.0	15.0	5.0	3.0	7.0	4.0	5.4	3.4	18.0	79.0	1.8	5.4	5.0	8.4	30.0	8.2	30.0	15.0	2.8	1.8
	0.01 M. KCl.																											

WORM		REACTION NUMBER 28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
A	0.1 M. KCl..																											
	H_2O........	1.8	7.8	2.8	2.0	6.8	2.8	2.8	2.0	11.0	2.8	3.0	1.8	1.8	2.2	1.8	2.4	2.0	1.8	1.8	2.0	1.8	2.0	2.4	1.8	3.4	2.0	2.8
B	H_2O........																5.4	3.8	1.8	2.0								
	0.1 M. KCl..	4.0	3.0	9.0	3.4	2.4	1.8	1.4	1.0	0.8	0.4	0.2	0.2	0.2	0.2	0.2					0.2	0.2						
C	0.01 M. KCl.	5.0	1.8	1.8	2.0	5.0	3.0	4.8	∞	3.8	3.0	16.0	∞															
	H_2O........													1.8	1.8	2.0	1.8	2.0	1.4	1.8	2.0	1.8	3.2	2.0	1.8	2.0	5.2	5.0
D	H_2O........	4.8	2.8									55.0	2.8	2.8	2.2	2.0	3.0	2.8	2.0	2.8								
	0.01 M. KCl.			70.0	1.8	42.8	4.8	∞	∞	∞	∞										1.8	2.0	3.0	∞	∞	1.8	∞	

WORM		REACTION NUMBER																									
		55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
A	0.1 M. KCl..		0.2	0.2					0.2												0.2	0.2	0.2				
	H_2O........	4.8			0.5	4.8	0.8	25.0		0.4	0.3	2.4	2.8	2.0	1.8	4.8	5.0	3.8	3.8	15.0				20.4	5.0		
B	H_2O........																										
	0.1 M. KCl..																										
C	0.01 M. KCl.			1.8	1.0	1.8	1.8	∞	6.2	∞	1.0	1.4	∞														
	H_2O........	2.2	5.4											4.8	5.0	1.8	1.2	5.0	3.8	7.8	3.0						
D	H_2O........	8.0	5.0	1.8	3.0	2.8	2.4	12.0	1.8	25.0	8.0	8.0	2.4													7.0	7.8
	0.01 M. KCl.													2.0	2.0	2.8	4.0	∞	1.8	2.0	∞	∞	2.0	2.8	∞		

SUMMARY

1. A new method for the determination of the effect of solutions on worms is described.

2. On comparing the efficiency of six potassium salts on the sensory cells of the dung-worm, Allolobophora foetida, it is found that the effect of the nitrate, chloride, acetate and sulphate is dependent on the amount of cations present, while in case of tartrate and citrate the amount of potassium ions is too small in all probability to attribute the effect to the cations.

3. On successive exposures of the worms to the potassium salt solutions of stronger concentrations (for example, 0.1 molecular potassium chloride), an initial acceleration in the reaction-time takes place. The reaction-times are at first long and become shorter and shorter until equilibrium is established, after which the reaction-time becomes long again.

4. On successive exposures of the worms to the weaker concentrations of potassium salt solutions (for example, 0.01 M. potassium chloride), to distilled water and to sucrose solutions, no initial acceleration in the reaction-times takes place.

5. When the worms in successive series are exposed to the distilled water at 33°C., while the room temperature is at 20°C., no acceleration in the reaction-time takes place, though the muscular activity of the worms increases considerably.

6. When a worm that has been stimulated by a salt solution to the point at which its reaction-time is shortest is transferred to a dish containing distilled water of the same temperature as that of the salt solution, its reactions are no longer nearly instantaneous, but are like those brought about by distilled water under ordinary conditions. But when the same worm is again transferred to the salt solution the very short reactions characteristic of the salt are at once resumed.

7. When the same method of procedure is applied in case of weaker concentrations of salt (for example, 0.01 molecular potassium chloride), into which the worms enter more frequently than into distilled water, the difference between the reactions in the salt solution and the distilled water is clearly marked.

8. The initial acceleration brought about on successive exposures of dung-worms to salt solutions is due to the specific action of the salts on the sensory cells.

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THE SECRETORY PRESSURE OF THE LIVER WITH SPECIAL REFERENCE TO THE PRESENCE OR ABSENCE OF A GALL BLADDER

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It has been determined that all of the extra hepatic ducts dilate after the removal of the gall bladder (1). This result seems to be due to interaction of the pressure exerted by the liver and the sphincter at the duodenal end of the common bile duct. It seemed desirable, therefore, to know whether the secretory pressure varied in species of animals with a gall badder from those without one. Our investigation is but one of several studies we have undertaken in reference to the function of the gall bladder.

The secretory pressure of the liver has been previously studied by several investigators. The earlier results obtained were quite variable. The most extensive and complete work on the subject was done by Herring and Simpson (2), who investigated both the secretory pressure of the liver and the process of bile absorption, after occlusion of the common bile duct. Their method of experimentation consisted in exposing the biliary tract with the animal under chloroform anesthesia and, after clamping the cystic duct, placing a cannula in the common bile duct, A vertical glass tube, having an internal diameter of 1 mm., was attached to the cannula. The lower end of the glass tube was adjusted so that the zero mark was at the level of the hepatic duct at the portal fissure. By means of a T-tube interposed between the cannula and the vertical tube, it was possible to record the rate of bile flow at zero pressure. Appropriate precautions were taken to keep the animal warm and in good condition. Many controls were taken in order to obtain trustworthy data. The maximum pressure to which the bile would rise in the tube was taken as a measure of the secretory pressure expressed in millimeters of bile. The pressure was measured in cats, dogs, rabbits, guinea pigs and monkeys. Their results may be briefly tabulated as follows:

1. The average of 18 cats was 304.4 mm. bile; maximum pressure 375; minimum pressure 211.
2. The average of 8 dogs was 300.0 mm. bile; maximum pressure 342; minimum pressure 243.
3. The average of 6 rabbits was 259.5 mm. bile; maximum pressure 306; minimum pressure 181.
4. The pressure in two guinea pigs was 122 and 154 mm. bile, respectively.
5. The pressure in two monkeys was 220 and 321 mm. bile, respectively.

The secretory pressure of the liver was measured also in cats and dogs by Mitchell and Stifel (3). They found the average pressure of liver secretion in cats to be practically the same as that obtained by Herring and Simpson.

We measured the secretory pressure of the liver in anesthetized and unanesthetized animals, and since we wished to determine whether there was any relation between the secretory pressure of the liver and the presence or absence of the gall bladder, only comparable species were used.

The secretory pressure of the liver in anesthetized animals. Our method of measuring the secretory pressure of the liver in anesthetized animals differed in only a few respects from that used by Herring and Simpson. The animals which had been fasted for about twelve hours previously were lightly etherized, and a cannula was inserted into the common bile duct with its point directed toward the liver. In the smaller species the insertion of the cannula was often quite difficult. An upright glass tube with an internal diameter of about 2 mm. was attached to this cannula and arranged so that its lower end was roughly in the plane passing through the middle of the liver. Therefore the lower end of the tube marked practically zero pressure. The maximum distance that the bile rose in this tube, expressed in millimeters of bile, was taken as a measure of the secretory pressure of the liver. The operative work was always performed with as little trauma as possible and the general condition of the animals, as regards temperature, light anesthesia etc., was always well maintained. In some experiments the cystic duct was left patent while in others it was either ligated or a small clamp applied to it.

In the beginning of an experiment the bile usually would rise quite rapidly in the tube, but as the pressure increased the flow became slower and finally stopped. Quite frequently, after reaching a maximum

height it dropped several millimeters before being maintained at a constant level. In some experiments the pressure was taken two or three times with an interval between. The maximum pressure, not including the respiratory rise, was always the one recorded.

The secretory pressure of the liver in animals having a gall bladder. The secretory pressure was measured by the method described in the species of animals having a gall bladder, as follows: guinea pig, rabbit, spermophile and goat.

Herring and Simpson report variable results in the guinea pig and the secretory pressure of the liver was rather low in their two successful experiments. Our results were fairly constant, and some of the pressures obtained were quite high (table 1).

Our results in the rabbit do not differ materially from those of previous investigators (table 1).

The secretory pressure of the spermophile (C. tridecemlineatus) was measured because it is fairly comparable in regard to size and habits with the rat, which does not possess a gall bladder. It was found to be quite difficult to obtain the pressure in this species probably because the liver was not able to secrete enough to fill the manometer with the animal under an anesthetic. We had many failures in this species. The pressure was found to be quite low (table 1).

The secretory pressure of the liver in the goat seems never before to have been measured. In our experiments it was found to be relatively low (table 1).

The secretory pressure of the liver of species of animals which do not possess a gall bladder. Only two species of animals normally without a gall bladder—the rat and pocket gopher—were found suitable for experimentation. In both these species the pancreatic duct empties directly into the bile duct and consequently a combined pressure of the two glands was measured. However, by ligating the pancreatic duct before measuring the pressure it was determined in a few experiments that the effect of this gland was negligible. We attempted to determine the pressure in the horse but found it technically very difficult; the same was true of the pigeon.

The secretory pressure in the rat was easy to obtain and quite high relatively (table 1). It was found difficult to obtain the secretory pressure in the pocket gopher (G. bursarius), and all the pressures were relatively low (table 1).

Secretory pressure of the liver in unanesthetized animals. In order to determine how much the anesthesia affected the results, a few measure-

TABLE 1

Secretory pressure of the liver in various species of animals. Animals etherized in each instance. Two species, the rat and pocket gopher, do not possess a gall bladder

SPECIES	NO.	WEIGHT	CONDITION	
		grams		
Rabbits	1	2000	Good	308
	2	2440	Good	225
	3	2275	Good	245
	4	2155	Good	290
	5	1765	Thin	255
		Average		264.6
Guinea pigs	1	775	Good	200
	2	707	Good	210
	3	480	Good	190
	4	540	Good	195
	5	755	Good	218
		Average		202.6
White rats	1	190	Good	225
	2	160	Good	200
	3	180	Good	215
	4	190	Good	225
	5	165	Good	225
	6*	165	Good	195
	7*	160	Good	220
		Average		215
Pocket gopher	1	270	Good	140
	2	225	Good	125
	3	325	Good	160
	4	170	Good	130
	5	270	Good	180
		Average		147
Striped gopher	1	140	Good	125
	2	135	Good	125
		Average		125
Goat	1	24.0	Fair	185
	2	10.4 kg.	Fair	180
		Average		182.5

* Pancreatic duct ligated.

ments of the secretory pressure were taken in unanesthetized dogs. A small metal cannula was sutured into the gall bladder, practically eliminating the capacity of this viscus, and the common bile duct was doubly ligated and sectioned. After the animal recovered from the operative procedure, the secretory pressure was measured in the same manner as in the anesthetized animal and it was found to remain very constant in the same animal from day to day. In the sleeping dog the pressure usually varied between 320 and 345 mm. Under certain experimental conditions it would go quite high, in one instance going as high as 550 mm. From these results it seems that the secretory pressure is usually higher when the animal is not under an anesthetic. In none of our own experiments nor in those recorded has the secretory pressure of the anesthetized dog been noted to be, as an average, quite so high as in most instances in which it was measured in the unanesthetized animal.

CONCLUSIONS

The secretory pressure of the liver was found to vary considerably in the various species of animals. The reason for this is not clear; there may be many causes; however, the presence or absence of the gall bladder does not seem to be one of them. The secretory pressure of the liver appears to be somewhat greater in unanesthetized animals than in those under an anesthetic but since the data obtained on anesthetized animals were only comparative, the conclusion that the presence or absence of the gall bladder bears no relation to the secretory pressure of the liver is justified.

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